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ROLE OF *SLC11A1* GENE IN DISEASE RESISTANCE

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Review paper

Abstract: Genetic improvement in livestock was achieved earlier by selective breeding of individuals with superior phenotypes. Now due to the advances in molecular genetics and biotechnology candidate genes of economic traits can be included in selection for breeding programmes. Genes responsible for the resistance/susceptibility to infections with various pathogens (Major Histo Compatibility *(MHC)* genes, Solute Carrier family11 member A1 *(SLC11A1)* gene, Toll Like Receptor *(TLR)* genes etc.), have been recently identified and characterized in human beings as well as in many animals . Among these the role of *SLC11A1* gene is very important due to its association with resistance/ susceptibility to various intracellular pathogens in human as well as in livestock species. The *SLC11A1* gene, formerly known as natural resistance-associated macrophage protein 1 *(NRAMP1)* encodes an integral membrane protein regulating the activity of macrophages. Genetic resistance/ susceptibility to diseases due to candidate gene polymorphisms could be used in selection and breeding for disease resistance in animals.

Key words: candidate gene, intra cellular pathogen, paratuberculosis, resistance, *SLC11A1*, susceptibility.

Introduction

The Solute Carrier family11 member A1 *(SLC11A1)* gene, previously known as Natural Resistance Associated Macrophage Protein 1 *(NRAMP1)* gene is 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)*. Since S*LC11A1* is associated with pH dependant transport of divalent cations (like Fe^{2+}, Mg^{2+} etc) through the phagosome membranes, are essential for many cellular functions. Experimental studies suggest that the transport is from the lumen of the phagolysosome to the cytosol, which prevents the acquisition of these

cations by intracellular pathogens *(Forbes and Gros, 2003)*. Under normal physiological conditions, *SLC11A1* gene delivers bivalent metal cations from the cytosol into acidic endosomal and lysosomal compartments where the Fenton and Haber-Weiss reaction generate toxic antimicrobial radicals for direct antimicrobial activity against phagocytosed microorganisms. *(Goswami et al., 2001)*. *SLC11A1* gene have pleiotropic effects on macrophage function, that include increased chemokine KC, tumour necrosis factor-α, interleukin-1β, inducible nitric oxide synthase and major histocompatability complex class II expression; all are important in resistance to intracellular pathogens *(Awomoyi, 2007)*.

There are so many experimental evidences regarding the association of *SLC11A1* gene polymorphisms with resistance to infectious diseases in human beings as well as animals. In mouse *SLC11A1* gene confers resistance to intracellular pathogens like Mycobacterium spp., Salmonella spp. and Leishmania spp. *(Vidal et al., 1995)*. The polymorphism of this gene and its association with disease resistance is widely studied in many species and important ones are reviewed here.

Role in human beings

Autoimmune Diseases and Cancer

The *SLC11A1* gene is located on human chromosome 2q35 contains 15 exons with a microsatellite polymorphism with a Z-DNA forming dinucleotide repeats in the 5' terminal region and it is associated with susceptibility/resistance to viral as well as protozoal infections. There are two predominant *SLC11A1* $(GT)_n$ Z-DNA promoter alleles $(GT)_5AC(GT)_9G$ known as allele 3, which drives high gene expression, while the allele $T(GT)_5AC(GT)_{10}G$ known as allele 2, drives low gene expression *(Searle and Blackwell, 1999)*. So the allele that drives high gene expression is associated with autoimmunity and cancer but protects against infectious diseases while the one that drives low expression is associated with infection such as tuberculosis, but protects against autoimmunity and cancer. These observations suggest that chronic hyper activation of macrophage associated with allele 3 functionally linked to cancer and autoimmune disease susceptibility. The poor level of *SLC11A1* gene expression promoted by allele 2 contributes to infectious disease susceptibility *(Awomoyi et al., 2007)*.

Tuberculosis

Tuberculosis is an urgent public health problem and the causative agent is *Mycobacterium tuberculosis*. The *SLC11A1* gene is a primary candidate for its association with tuberculosis susceptibility. In a case control study of 329

tuberculosis patients and 324 control subjects, the association between allele 2 of a functional *SLC11A1* gene polymorphism (by regulation interleukin -10) and tuberculosis was evaluated *(Awomoyi et al., 2002)* and the role of *SLC11A1* in host defense mechanism and development of tuberculosis was studied by *Li et al. (2011)*. *Jin et al. (2009)* reported that the frequency of variant genotypes (TGTG+/delete and TGTG delete/delete) was significantly higher in the pediatric tuberculosis group than in the control group at the 3'UTR locus and did not identify any statistically significant differences between the tuberculosis and control groups with regard to the frequency of genotype variants G/C and C/C at the INT4 locus and differences in genotype distribution at the 3'UTR locus were only identified in female subjects, with a greater number of variant genotypes in the pediatric tuberculosis group.

Rheumatoid arthritis

Rheumatoid arthritis is a chronic inflammatory joint disease. *Yang et al. (2000)* was conducted a study to determine whether NRAMP1 polymorphisms are associated with susceptibility to rheumatoid arthritis in Koreans and nine NRAMP1 polymorphisms (1 microsatellite, 1 variation in 3' UTR, 5 silent substitution, 2 amino acid substitution) were typed by PCR-RFLP in 74 patients with rheumatoid arthritis and 53 healthy controls in Koreans. They found that three NRAMP1 polymorphisms (823C/T, D543N and 1729+55del4) were significantly associated with rheumatoid arthritis. *Singal et al. (2000)* examined the role of NRAMP1 gene polymorphisms in susceptibility to rheumatoid arthritis and showed that variation at position 543 in exon 15, which involves substitution of negatively charged aspartic acid (D) by uncharged asparagine (N), and the deletion of TGTG in the 3' UTR may confer protection from development of rheumatoid arthritis.

Role in animals

Tuberculosis

Bovine tuberculosis is caused by *Mycobacterium bovis* is a considerable health hazard to animal keepers and general communities. A microsatellite polymorphism within the *SLC11A1* gene (allele 211, allele 215 and allele 217) is significantly related to lower incidence of bovine tuberculosis in Chadian cattle (African Zebu) *(Kadarmideen et al., 2011).*

Brucellosis

Brucellosis is a major zoonotic infection worldwide. In cattle the causative agent is *Brucella abortus. Adams and Templeton* (*1998*) reported a $(GT)_{13}$

microsatellite allele at 3'UTR of *SLC11A1* gene has a significant association with natural resistance to brucellosis. Acccording to *Barthel et al. (2001)*, $(GT)_{13}$ microsatellite allele at 3'UTR of *SLC11A1* gene restrict the intracellular replication of Brucella organisms, this allele either in homozygous (GT_{13}/GT_{13}) or in heterozygous (GT_{13}/GT_n, where n=14,15 or16) is significantly ($p<0.01$) associated with improved macrophage function in buffalo, by increased production of hydrogen peroxide (H_2O_2) and nitrous oxide (NO). *Capparelli et al. (2007a, 2007b)* reported a significant association of polymorphismsat 3′ UTR of SLC11A1 gene with resistance/susceptibility to brucellosis in buffalo (*Bubalus bubalis*). A significant association ($p < 0.001$) was found between the *B. abortus* macrophage in vitro killing assay phenotypes and the bovine *SLC11A1* 3'UTR genotypes, which suggests that the A allele may be associated with resistance (*Martinez et al., 2008*). *Ganguly et al., (2008)* demonstrated that macrophages from $(GT)_{13}$ buffaloes produced more nitric oxide and H2O2 when challenged with brucella LPS.

But on the contrary to these findings, a study conducted by *Kumar et al. (2005)* reported lack of association of brucellosis resistance with $(GT)_{13}$ microsatellite allele even at homozygous condition in *Bos indicus* and crossbred cattle *(Bos indicus x Bos taurus)*. Again, *Paixao et al. (2007)* did not detect association between a 3'UTR polymorphism of *SLC11A1* gene and resistance to brucellosis in cattle. *Kumar et al. (2011)* identified *AluI* and *TaqI* polymorphisms in and around TM4 (transmembrane domain 4) of *SLC11A1* gene in cattle but an association of the observed allelic variants with the resistance/susceptibility to brucellosis could not be established.

Paratuberculosis/Johne's Disease

Paratuberculosis also referred s Johne's disease is a contagious bacterial disease. The intracellular bacteria, *Mycobacterium avium ssp paratuberculosis* (MAP) is responsible for paratuberculosis and causes diarrhea, reduced milk production, reproductive failure, weight loss and death. In livestock a number of candidate genes were studied and selected on the basis of their association to mycobacterial diseases, like *SLC11A1*, Toll Like Receptor *(TLR)*, Caspase Associated Recruitment Domain 15 *(CARD 15)*, Major Histocompatibility Complex *(MHC)* and Cytokines (interleukin-10 and interferon gamma) and their receptors.

Reddacliff et al. (2005) identified *SLC11A1* gene polymorphisms in two phenotypically defined Merino flocks with a high prevalence to MAP infection and possible associations with susceptibility/ resistance to Johne's disease were detected. *Liandris et al. (2009)* sequenced the caprine *SLC11A1* gene (GeneBank FJ388877) and investigated the potential association of its polymorphisms with test positivity of goats to MAP infection. In a similar study conducted in goats in

Greece, it was observed that the 3'UTR of caprine *SLC11A1* gene contains two microsatellites with a variable number of guanine-thymine (GT) repeats named region A and B and statistically significant association was established between genotypes of region B and ELISA (Enzyme Linked Immuno Sorbent Assay) results of paratuberculosis, whereas the presence of B_7 allele was found to contribute to ELISA negativity *(Korou et al., 2010). Vacca et al. (2011)* investigated the chromosomal location of the caprine *SLC11A1* gene, the genomic regions corresponding to the Sarcoma Homology 3 (SH3) binding motif (in exon 2), the glycosylation site (in exon 10), exon 15 and the partial 3'UTR region and the genetic variability of the 3'UTR microsatellite in six dairy goat breeds.

A study conducted by *Ruiz-larranaga et al. (2010)* suggests that SNP c.1067C > G of *SLC11A1* gene may be a potential causal variant that causes an amino acid change in codon 356 from proline to alanine (P356A) that could alter *SLC11A1* protein function, although functional studies are needed to assure this point and this association study supports the involvement of *SLC11A1* gene in susceptibility to MAP infection in cattle.

Salmonellosis

So far only a few candidate genes or regions with an association for differences in Salmonella resistance or ability to withstand the consequences of the infection have been identified in chickens. *SLC11A1* gene showed a significant association on early resistance to Salmonella *(Hu et al. 1997; Liu et al. 2003).*

Conclusion

The most important diseases in which genetic selection can be applied include mastitis, bovine leukaemia, gastro intestinal parasitism, tuberculosis and paratuberculosis in cattle, paratuberculosis, gastrointestinal parasites and foot rot in sheep and goats. Disease is the most important constraint in the animal production system. So the selection of animals for increased genetic resistance to diseases will lead to the production of a more healthy and productive stock. If specific alleles associated with resistance or susceptibility to diseases were identified, then animals possessing favourable alleles or not having unfavourable alleles could be used for further breeding programmes. Further studies are required in the area of identification of candidate gene polymorphisms associated with disease resistance/ susceptibility and inclusion of these markers in selection and breeding programmes.

Uloga *SLC11A1* gena u otpornosti na bolesti

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Rezime

Genetsko unapredjenje u stočarstvu je ranije ostvarivano selekcijom pojedinih grla sa superiornim fenotipovima. Danas, kao rezultat napretka u molekularnoj genetic ii biotehnologiji, kandidat geni osobina od ekonomske važnosti se mogu uključiti u selekciju u odgajivačkim programina. Geni odgovorni za otpornost/osetljivost na infekcije koje izazivaju različiti patogeni (Major Histo Compatability (*MHC*) geni, Solute Carrier familija11 član A1 (*SLC11A1*) gen, Toll Like Receptor (*TLR*) geni, itd.), su od skora identifikovani i uradjena je njihova krakterizacija kod ljudi, kao i kod mnogih životinjskih vrsta. Od navedenih, uloga *SLC11A1* gena je veoma važna zbog njegove povezanosti sa otpornošću/osetljivošću na različite medjućelijske patogene kod ljudi kao i kod životinjskih vrsta. *SLC11A1* gen, ranije poznat kao prirodni makrofag protein 1 koji je povezan sa otpornošću (*NRAMP1*) kodira protein integralne membrane koji reguliše aktivnost makrofaga. Genetska otpornost/osetljivost na bolesti zbog polimorfizama kandidat gena bi se mogla koristiti u selekciji i odgajivanju za razvoj otpornosti životinja na bolesti.

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