Review Article

Cloning and Polymorphism of Disease Resistance SLC11A1 Gene in Pig (Sus scrofa): A Review

Bhanita Devi[1*], S. Laskar[2], P. Borah[3] and P. K. Bharti[4]

[1]Department of Animal Biotechnology, LUVAS, Hisar-125004, Haryana, INDIA
[2]Department of Animal Genetics and Breeding, CVSc, Khanapara-781022, Assam, INDIA
[3]Department of Animal Biotechnology, CVSc, Khanapara-781022, Assam
[4]LPM, ICAR-IVRI, Izatnagar-243122, Bareilly, Uttar Pradesh, INDIA

***Corresponding author:** drbhanita@gmail.com

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Abstract

Infectious diseases are responsible for major economic losses in livestock production. Using disease resistant breeds is of considerable importance in livestock species. Solute carrier family 11member 1 (SLC11A1) which is also called as Natural Resistance-Associated Macrophage Protein1 (NRAMP1), is one of the most potent candidate genes conferring host's genetic resistance to various antigenically different intracellular pathogens. The gene controls the exponential growth of the bacteria during the early phase of infection. SLC11A1 have been identified and cloned in pigs and it is strongly expressed on macrophages and neutrophils following stimulation with lipopolysaccharide. The present review discuss the localization, function and molecular techniques used to study the cloning and sequencing of SLC11A1 gene polymorphism in pigs by using Restriction Fragment Length Polymorphism (PCR-RFLP) and Single Strand Conformation Polymorphism (PCR-SSCP) methods as the SLC11A1 gene plays a very important role due to its association with resistance/ susceptibility to various intracellular pathogens in human as well as in livestock species.

Key words: Cloning, *NRAMP*1, PCR-RFLP, PCR-SSCP, Pig, Polymorphism, *SLC11A1*

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Introduction

The livestock sector alone contributes nearly 28.5% of value of output at current prices of total value of output in Agriculture, Fishing and Forestry sector. The overall contribution of livestock sector in total Gross Domestic Product (GDP) is nearly 4.5% at current price (Annual Report, 2016-17). Infectious diseases in India causes annual loss mainly through morbidity, mortality, reduced fertility, production losses and inefficient feed utilization. Diseases in animals have numerous impacts such as loss of productivity, loss of

income from activities using animal resources, prevention or control costs and suboptimal use of production potential (McInerney, 1996). According to 19th Livestock Census (2012), the total livestock population and the total pig population in the country has decreased by about 3.33% and 7.54%, respectively over the previous census. The total pig population in the country is 10.29 million which accounts for only 2.01% of the total livestock population (512.5 million). Susceptibility to the infectious diseases depends more or less on genetic component of the animals. Infectious diseases are difficult to cure and cause significant economic losses in livestock species. Selective breeding is used to increase resistance against infectious disease and it may prove to be an economical and sustainable practice. Genetic methods, such as selection of disease resistance in the pig, have not been widely used. Genetic resistance or susceptibility to diseases due to candidate gene polymorphisms could be used in selection and breeding programme for disease resistance in animals (Thomas and Joseph, 2012). In livestock, a number of candidate genes has been studied and selected on the basis of their association to resistance or susceptibility in certain other diseases and their known role in disease pathogenesis. These genes include solute carrier family 11 member 1 (*SLC11A1*), interferon gamma (IFN-γ), peptidoglycan recognition protein 1 (*PGLYRP-1*), Toll-like receptors (*TLR*s), Caspase associated recruitment domain 15 (*CARD*15), mannose binding lectin-1 (*MBL*-1), Nitric oxide synthase (NOS_2) etc. Genome-wide association studies have attempted to confirm associations found and identify new genes involved in pathogenesis and susceptibility. Selection of disease resistance animal for breeding might be the prophylactic measure of first choice. Breeding stock could be chosen by marker-assisted selection (MAS) independently from environmental factors because of development of a genetic marker for disease resistance (Meuwissen and Van Arendonk, 1992). Molecular marker is a potential selection tool for disease resistance and susceptibility in animals. The completion of the porcine whole genome sequencing has provided powerful tools to identify the genome regions that harbor genes controlling disease or immunity (Zhao *et al*., 2012). Presently, many types of molecular markers have been utilized to detect the variation among individual and population. The Solute Carrier family 11member 1 (*SLC11A1*) is a proton-coupled divalent metal ion transporter which was previously known as Natural Resistance-Associated Macrophage Protein1 (*NRAMP1*). It is also involved in the innate immune response against pathogens of viral, bacterial and protozoan origin (Awomoyi, 2007).The *NRAMP1*was first isolated in the mouse as a candidate gene for the Bcg/Ity/Lsh locus that conferred genetic resistance/susceptibility of inbred mouse strains to infection by *Mycobacteria*, *Salmonella*, and *Leishmania* (Vidal *et al*., 1993). Belouchi *et al*. (1995) reported that the members of *NRAMP* gene family were first found in mouse and then Belouchi named these genes as the *NRAMP1*gene family. The *SLC11A*1 gene was originally recognized in the mouse as three loci Ity, Lsh, and Bcg and the latter proved to be the *NRAMP1*, which has been recently renamed as *SLC11A1* (Vidal *et al*., 1996).Transgenic mice homozygous for engineered mutations in *NRAMP1* have similar phenotypic effects on natural resistance to intracellular

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pathogens which indicates a critical role of *NRAMP1* protein in pre-immune macrophage function (Vidal *et al*., 1995a). A naturally occurring Gly→Asp mutation at amino acid 169 of *SLC11A1* makes mice as susceptible to *Leishmania donovani*, *Salmonella typhimurium* and *Mycobacterium bovis* as gene-disrupted mice (Vidal *et al*., 1995b). The amino-terminal domain of *NRAMP1* binding to microtubules that could mediate microtubule-dependent phagosome and/or lysosome transport (Kishi *et al*., 1996). The *NRAMP1* encodes phospho-glycoprotein with whole membrane and has the characteristics of transport activity and ion channel (Vidal *et al*., 1996). *NRAMP1* acts by stabilizing the mRNA of genes associated with macrophage activation, thus accounting for the functional differences that have been attributed to these macrophage populations (Brown *et al*., 1997). *NRAMP1* gene encodes a protein with 12 trans-membrane domains that localizes in the phagolysosome membrane, particularly in macrophages (Gruenheid *et al*., 1997). The whole sequence of *NRAMP1* in swine encodes 539 amino acids, which has 87% similarity with human beings (Tuggle *et al*., 1997). A link was confirmed *in vivo* between iron metabolism and *NRAMP1* function and established that excess iron hampers *NRAMP1*- encoded protein function and strongly argue for a role of *NRAMP1* as an iron pump that depletes the phagosomal compartments of this nutrient, leading to starvation of the pathogen of this essential cation (Gomes and Appleberg, 1998).

NRAMP1 protein coding sequences and gene structures showed a high degree of conservation across species which suggested that the protein cannot tolerate significant alteration in primary structure (Dorschner and Phillips, 1999; Zhang *et al*., 2000).The movement of cations may actually occur in the opposite direction which results an increased concentration of iron into the phagolysosome and may favour bacterial killing by generating oxygen intermediates through the Fenton reaction (Goswami *et al*., 2001).The *NRAMP* protein has a pH dependent cation transport activity and they act as a transporter of divalent cations like Fe^{2+} and $Mn^{2+}$from the lumen of the phagolysosome towards the cytosol, thereby preventing acquisition of iron by intracellular pathogens (Wyllie *et al*., 2002; Forbes and Gros, 2003). Polymorphisms of the human *NRAMP*1 gene are significantly associated with pulmonary *Mycobacterium avium* complex infection (Tanaka *et al*., 2007), Crohn's disease (Gazouli *et al*., 2008), rheumatoid arthritis (Ates *et al*., 2009), type 1 diabetes (T1D) (*Yang et al*., 2011).

Localization of *SLC11A1* Gene

SLC11A1 localizes to membranes of late endosomes and lysosomes in mammals but not to early endosomes (Gruenheid *et al*., 1997; Searle *et al*., 1998) and suggests that it targets directly from the trans-Golgi network. *NRAMP1*mainly exists in reticulo-endothelial cells and organs such as outer white blood cells, spleen, and lung which can resist several intracellular pathogenic microorganisms (Blackwell, 1996). *NRAMP1*gene in swine is located on q23-26 of chromosome 15 (Sun *et al*.,1998) and the whole length of *NRAMP1* in swine is about 15 kb which contains15 exons and 14 introns as human beings and mouse

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(Govoni *et al*.,1995; Marquet *et al*., 2000; Wu *et al*., 2007). It was reported that the whole sequence of *NRAMP1*in swine encodes 539 amino acids, which had 87% similarity with human beings (Tuggle *et al*., 1997). *NRAMP1*gene is specially expressed in phagocytic cells, such as macrophages neutrophilic granulocytes and outer blood cells.

Functions of *SLC11A1* Gene

NRAMP1 functions as an H1-sensitive divalent cation transporter in the membrane of intact phagosomes *In situ* as they have the ability to transport metals because of their expression by both phagocytes and pathogens. *NRAMP* proteins appear to play a key role in the host–parasite interface in the microenvironment of the phagosome (Forbes and Gros, 2003). *SLC11A1* is a divalent cation (Fe2+, Zn2+ and Mn2+) transporter (Goswami *et al*., 2001) and an antiporter that can flux divalent cations in either direction against a proton gradient. *SLC11A1* in late endosomes/ lysosomes delivers divalent cations from the cytosol to the acidic compartment.

Cloning and Sequencing of *SLC11A1* Gene in Pig

The cloning and sequencing of a full-length *SLC11A1* gene cDNA was done and found that the entire protein coding region of the pig *NRAMP1* gene which is corresponding to a mouse protein is known to cause susceptibility to infection by several different bacteria. Tuggle *et al.* (1997) reported that pig protein encoded within the gene is highly similar to the mouse and human *NRAMP1* and new *NRAMP1* sequence identified a 538 amino acid protein. The derived pig protein sequence had much higher identity to *NRAMP1* from humans, cattle, and mice (87%, 88% and 85%, respectively) than to *NRAMP2* from humans or mice (64% and 62%, respectively). Expression profile of pig *NRAMP1* indicates that it is expressed in spleen which is a rich source of immune cells and may be expressed in other tissues at low levels. Their data strongly indicates that the newly cloned gene has a similar physiological function in pigs to that seen for mouse *NRAMP1*. It was revealed that the association of *NRAMP1* to *Salmonella* infection in pigs can be tested from their information (Tuggle *et al*., 1997).

The cloning of the full-length cDNA for porcine *NRAMP1*suggest that over 85% identities in amino acid sequence to its congeners from humans, mice, cattle and sheep. Expression of porcine *NRAMP1* mRNA was cell and tissue specific and was highest in macrophages. Investigation of the molecular mechanisms by which *NRAMP1* is induced showed that Lipopolysaccharide(LPS) -induced expression in macrophages, neutrophils and peripheral blood mononuclear cells was time and dose dependent and was mediated primarily through Cluster of Differentiation (CD) -14. Induction of *NRAMP1* required de novo protein synthesis and Mitogen-Activated Protein Kinases (MAPK) as essential factors. Blockage of either p38 or p42/44 MAPK pathways suppressed the expression of *NRAMP1* to basal levels. Their findings suggest that

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bacterial infection and pro-inflammatory mediators induce *NRAMP1* expression via activation of MAPK pathways. It was revealed that cloned porcine *NRAMP1* cDNA and characterized tissue expression patterns and molecular mechanisms of induction of *NRAMP1* by LPS, TNF-a and IL-1b and shown that LPS-induced *NRAMP1* induction in macrophages is primarily dependent upon CD14 which also involves newly synthesized protein(s). It was reported that p38 and p42/44 MAPK cascades are involved in the induction of *NRAMP1* expression in response to LPS, Tumor Necrosis Factor (TNF) -a, and Interleukin (IL)-1b. The relative significance of other protein kinases, such as PKC, protein kinase A, G proteins, and ceramide-activated protein kinase, in *NRAMP1* induction remains to be elucidated (Zhang *et al.,* 2000).

Devi *et al*. (2016) studied the genetic diversity of the *SLC11A1* gene in Doom pigs of Assam especially on its evolution and differentiation within and among species, the partial sequence of the *SLC11A1* gene was sequenced, characterized and compared with published sequences of pigs and other livestock species. The gene sequence of Doom pigs showed the highest sequence identity with EF200584.1 (exotic pig) and the lowest similarity AY368475.1 (large white strain 008). One single nucleotide polymorphism (SNP) was identified in the heterozygous sequence at the 736 bp position (A→G). The sequence showed the highest sequence identity (81.74%) with that of *O. aries* and the lowest similarity (39.12%) with *B. bubalis* (Mehsana breed), respectively. Phylogenetic analysis revealed that the Doom pig is more closely related to EU135795.1 (Chinese local pig) and EF200584.1 (Pig, Iowa State University). The sequencing and phylogenetic analysis of the *SLC11A1* gene of Doom pigs could be used for genetic selection with disease-resistance varieties and upgradation of indigenous germplasm of domestic livestock.

The sequence analysis of the *NRAMP*1 gene in Tibetan, Gansu Black, Large White, Yorkshire, and Duroc pig breeds revealed the presence of 11 nucleotide variants in intronic regions, 2 nucleotide variants in the control region, 10 nucleotide variants and one deletion in the 3' non-coding region and 15 nucleotide variants in the exons. Only 4 nucleotide variants resulted in amino acid changes. The Tibetan pig *NRAMP1* protein harbors 10 transmembrane domains. The analysis also predicted 10 serine phosphorylation sites, 3 threonine phosphorylation sites, and 4 tyrosine phosphorylation sites in the *NRAMP*1 protein of Tibetan pigs. Predictions of the Tibetan pig *NRAMP*1 tertiary structure revealed 7 putative a -helices and 5 putative b-sheets. Predictions of the Yorkshire pig *NRAMP*1 protein revealed significant differences compared with the Tibetan pig *NRAMP*1 protein. The differences in transmembrane domains and tertiary structures of the *NRAMP*1 protein of the Tibetan and Yorkshire pig breeds could explain differences in the disease resistance of these two breeds (Wang *et al.,* 2017).

PCR-RFLP to Detect Polymorphism of *SLC11A1*Gene

Mapping of *NRAMP1* in the pig as potential candidate gene in controlling pig resistance to *Salmonella* infection was reported (Sun *et al.,* 1998). It was revealed that the primers from the pig cDNA to amplify a

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1.6 kb fragment between exons 1 and 3. Pig-rodent somatic cell hybrid panel was used to map the *NRAMP1* pig chromosome 15 (SSC15) with 100% probability and the regional assignment was SSC15q23-26 with 87% concordance. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) marker was developed by using the *HinfI* enzyme and three alleles were identified from a population including 11 breeds. Linkage analysis confirmed the physical assignment by using the PiGMaP reference families. Pig *NRAMP1* was linked to SSC15 markers S0088, S0149 and S0284 (LOD > 3). A small population study revealed large allele frequency differences among tested breeds. An A allele is only observed in dam (white) lines whereas a similar exclusivity of the C allele was seen in sire (coloured) breeds.

Analysis of the variation at a number of genetic loci using PCR-RFLP in two local Romanian pig breeds, the Mangalitsa and Bazna was studied (Ciobanu *et al*., 2001). These breeds are part of a conservation programme in Romania and marker information may be useful in preserving a representative gene pool in the populations. Mangalitsa has a higher frequency of allele A (0.33), although allele *B* is the most common in both breeds. Allele *B* was the most prevalent and polymorphism was assessed at loci which are known to cause phenotypic variation, potentially involved in trait differences or putative candidate genes. The traits considered are disease resistance, growth, coat colour, meat quality and prolificacy. Even though the populations are small and the markers are limited to specific genes, significant differences in five of the ten characterized loci were reported. In some cases the observed allele frequencies were interesting in relation to gene function and the phenotype of the breed.

Yan *et al*., 2004, investigated the genetic variations in a 1.6 kb region spanning exon 1 and exon 3 of the porcine *NRAMP1* gene by PCR-*Hinf*I -RFLP in samples of 1347 individuals from 21 Chinese indigenous pig populations and 3 western pig breeds. Three alleles (A, B and C) and four genotypes (AA, BB, AB and BC) were detected. The differences in genotype and allele frequencies between Chinese indigenous pig populations and exotic pig breeds were found to be significant, while in general the differences in genotype and allele frequencies within the Chinese indigenous pig populations were not significant. The allele C was detected only in Duroc, Leping Spotted and Dongxiang Spotted pig and the two Chinese pig populations showed similar genotype and allele frequencies. Four Chinese Tibetan pig populations displayed genetic differentiation at the *NRAMP1* gene locus. In addition, intron 5 of the *NRAMP1* gene was isolated and characterized directly by sequencing the PCR products encompassing intron 5. The alignment of intron 5 of the porcine, human, equine and ovine *NRAMP1* gene showed similarity of 45.38% between pig and human, 52.55% between pig and horse, 63.47% between pig and sheep, respectively. Wu *et al*. (2008) designed to study the association of polymorphism of *NRAMP1* with some immune function and the production performance in Large White pig. The PCR-RFLP technique was applied to analyze the correlation between the polymorphisms of *NRAMP1*gene and immune function value of Polymorpho-

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nuclear Leukocytes (PMN) obtained by Nitroblue Tetrazolium (NBT) Reduction and effect of Cytotoxin in Monocyte and production performance in 165 Large White pigs. It was found that one *Nde*I restriction locus in Large White pig and both values of PMN by NBT Reduction and effect of Cytotoxin in Monocyte in genotype BB were higher than those in genotype AB ($P<0.05$) and the weight of 180-day-old pigs with genotype BB was higher than that with genotype AB ($P<0.05$). It was concluded that there was a significant correlation between different genotypes of *NRAMP1*gene and Immune function and production performance and it can be regarded as a candidate gene of disease resistance. It was also suggested that all those results provide valuable reference to further studies of pig disease resistance (Table 1).

Table 1: Restriction enzymes, polymorphism sites, genotypes, SNP identified and amino acid change in PCR-RFLP to detect polymorphism in SLC11A1 gene

S. No.	Restriction Enzyme	Polymorphism site	Genotype	SNP identified	Amino acid changes	References
1	*HinfI*	-	3 allele	-	-	Sun *et al.* (1998)
2	*HinfI*	-	A,B,C (Allele)	-	-	Ciobanu *et al.* (2001)
3	*HinfI*	Intron 5	AA, BB, AB and BC	No SNPs	-	Yan *et al.* (2004)
4	*Nde*I	Intron 6 (483 bp)	AA, AB and BB	-	-	Wu *et al.* (2008)
5	*Sma*I	Intron 1(738 bp position)	GG, AG and AA	A→G transition	-	Liu *et al.* (2011)
6	*Sma*I	One SNP at intron 1 and one SNP at exon 1	CC, CT, TT, AA, AG and GG	C>T and A>G		Ding *et al.* (2014)
7	*Sma*I	736 bp	AA, AB and BB	A↔G transition	-	Devi *et al.* (2015)

In Chinese indigenous pigs and exotic pigs one single nucleotide polymorphism (SNP) was found in intron 1 after digestion with *Sma*I and found A→G transition at 738 bp position (Liu *et al.*, 2011). The three genotypes; GG genotype: 506 bp, 232 bp and 198 bp, AG genotype: 506 bp 430 bp and 232 bp and AA genotype: 505 bp and 430 bp were reported in Landrace pigs. They could establish an association of polymorphism of the *SLC11A1* gene with the level of monocyte ($p=0.010$) and $CD4^{-}CD8^{+}$ percentage ($p = 0.041$) and also found that the animals with AA genotype had significantly ($p<0.05$) higher monocyte and $CD4^{-}CD8^{+}$ percentage than that of animals with GG and GA genotype monocyte. The T lymphocyte found at A→G transition site 738 in intron1 forms another restriction site for *Sma*I enzyme. It was suggested that the *SLC11A1* could be used as a marker gene for genetic selection of disease susceptibility in pigs.

To evaluate the effects of *NRAMP1* gene on immune capacity in pigs, tissue expression of NRAMP1 mRNA was observed by real time quantitative polymerase chain reaction (PCR) and the results revealed NRAMP1

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expressed widely in nine tissues. One single nucleotide polymorphism (SNP) (ENSSSCG00000025058: g.130 C>T) in exon1 and one SNP (ENSSSCG00000025058: g.657 A>G) in intron1 region of porcine *NRAMP1* gene were demonstrated by DNA sequencing and PCR-RFLP analysis. A further analysis of SNP genotypes associated with immune traits including white blood cell (WBC), granulocyte, lymphocyte, monocyte (MO), rate of cytotoxin in monocyte (MC) and CD4/CD8 T lymphocyte subpopulations in blood was carried out in four pig populations including Large White and three Chinese indigenous breeds (Wannan Black, Huai pig and Wei pig). The results showed that the SNP (ENSSSCG00000025058: g.130 C>T) was significantly associated with level of WBC % ($p = 0.031$), MO% ($p = 0.024$), MC% ($p = 0.013$) and $CD4^{-}CD8^{+}$ T lymphocyte ($p = 0.023$). The other SNP (ENSSSCG00000025058: g.657 A>G) was significantly associated with the level of MO% ($p = 0.012$), MC% ($p = 0.019$) and $CD4^{-}CD8^{+}$ T lymphocyte ($p = 0.037$).These results indicate that the *NRAMP1* gene can be regarded as a molecular marker for genetic selection of disease susceptibility in pig breeding (Dinga *et al*., 2014).

The population of Doom pigs was found to be polymorphic in respect of *SLC11A1* gene having 2 alleles with frequencies 0.8364 and 0.1636 for 'A' and 'B', respectively (Devi *et.al.*, 2015). It was revealed that the genotypic frequencies to be 0.71, 0.26 and 0.03 for AA, AB and BB genotypes, respectively.

PCR-SSCP to Detect Polymorphism

PCR-SSCP method was used to analyze the *NRAMP1* gene (exon 2 and intron 6) polymorphisms in Hezuo pig (Juan *et al.*, 2010). It was aimed to get molecular genetics information and provided the scientific basis for molecular marker of disease resistance of Hezuo pig. The result of PCR-SSCP polymorphism examination of the *NRAMP*1 exon 2 from cloning and sequencing indicated that there were two polymorphic loci 62 T→C and 92 A→G and this mutation did not induce the changes of encoding amino acid being controlled by allelic genes A and B. There were three genotypes, namely AA, BB and AB in *NRAMP1* exon 2. Frequency of A allele was 0.6519 which was found predominantly. The result of PCR-SSCP polymorphism examination of the *NRAMP1* intron 6 from sequencing analysis showed that ten new nucleotide polymorphisms in intron 6, which were the single nucleotide substitution of C→T at the position of 99, 225, 304, 307, 313, A→G at the position of 105, C→A at the position of 190, T→G at the position of 298, T→C at the position of 306 and 295-296 bp was detected one insertion: AA and CC genotype -→T, BB genotype → G. Among the five genotypes, namely AA, AB, BB, AC and CC; allele B was found predominant. From the statistical results it was revealed that the *NRAMP1* exon 2 SNPs sites in Hezuo pig was at Hardy-Weinberg disequilibrium ($P < 0.05$), PIC was intermediate polymorphism; *NRAMP1* intron 6 SNPs sites in Hezuo pig was at Hardy-Weinberg equilibrium ($P > 0.05$), PIC was high polymorphism (Table 2).

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Table 2: Restriction enzymes, polymorphism sites, genotypes, SNP identified and amino acid change in PCR-SSCP to detect polymorphism in SLC11A1 gene

S. No.	Restriction Enzyme	Polymorphism site	Genotype	SNP identified	Amino acid changes	References
1	-	Exon 2	AA, BB and AB	Two polymorphic loci 62 T→C and 92 A→G	No changes of amino acid	Juan *et al.* (2010)
2	-	Intron 6	AA, AB, BB, AC and CC	C→T,A→G, C→A,T→G, T→C,one insertion: AA and CC genotype -→T, BB genotype → G.	-	Juan *et al.* (2010)
3	*Sma1*	Intron 1	2 allele	A «G transitions	-	Tuggle *et al.* (2005)
4	*Nde*I	278-279 base sites	-	CA-TG mutation (40polymorphis, 6 of which were reported to be located in exons and 34 in introns)		Wu *et al.* (2007)

*NRAMP1*gene as candidate genes for contributing to resistance in *Salmonella cholera suis* (SC) challenge in pigs was investigated and reported five *NRAMP1* sequence differences polymorphisms and SNPs (Tuggle *et al.,* 2004). The effects these polymorphisms have on resistance to infection were tested in two experimental disease studies. In study 1, results showed *NRAMP1* genotypes are associated with decreased fecal bacterial load during infection (P values: < 0.0006 to < 0.06). In the second study, many additional immune traits and spleen and liver bacterial counts were collected. The *NRAMP1* genotypes were associated with bacterial count in liver (P < 0.05 and P < 0.0006) and with polymorpho-nuclear phagocytes (P < 0.003 to < 0.05). These data indicate *NRAMP1* gene variation may control, in part, response to *Salmonella* infection in pigs, and that these differences could be used to identify resistant animals. Tuggle *et al.* (2005) invented a method for determining improved innate immunity, disease resistance or performance in animals.

The method involves assays for a genetic difference in the *NRAMP1* gene of the animal. Novel *NRAMP1* sequence, assays and compositions for identifying the presence or absence of the alleles was provided and further two alleles in intron 1 upon Sma1 digestion were reported. It was reported that at A ↔G transitions, both the allele has G position at 737 and allele 7 also has A position at 737. The investigation of the porcine response to gastrointestinal infection with *Salmonella enteric* serovars *Cholerae suis* (narrow host range) and *Typhimurium* (broad host range) was also studied which revealed markedly different transcriptional profiles (Uthe *et al.,* 2007). Identification of seven genes through suppression subtractive hybridization as up-regulated in the mesenteric lymph nodes at 24 h post-inoculation (p.i.) in serovar *Cholerae suis*-infected

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pigs (ARPC2, CCT7, HSPH1, LCP1, PTMA, SDCBP, VCP) and three genes in serovar *Typhimurium*-infected pigs (CD47/IAP, CXCL10, SCARB2) were analyzed by real-time PCR at 8 h, 24 h, 48 h, 7 days (d) and 21 d p.i. A comparison between the two *Salmonella* infections revealed significant differences in transcriptional induction early in the infection (8–24 h) for the serovar *Typhimurium*-infected pigs, whereas the serovar *Cholerae suis*-infected pigs exhibited significantly higher levels of gene expression at the later time points (48 h–21 d), except for HSPH1. A similar gene expression trend was observed for immune-related genes involved in innate immunity and the inflammatory T helper 1 (Th1) response. Initial repression of gene expression in the serovar *Cholerae suis*-infected pigs from 8 to 48 h p.i. (*IFNG, IL12A, IL4, IL8* and *CSF2*) coincided with extended transcriptional activation throughout the 21 d infection (*IFNG, INDO, SOCS1, STAT1, IL1B, IL6, IL8* and *SLC11A1*). The serovar *Typhimurium*-infected swine presented a more transient induction of immune-related genes *(IFNG, INDO, IRF1, SOCS1, STAT1, IL1B, IL8, SLC11A1*) early in the infection (24–48 h) followed by a significant repression of *IL12A, IL12B, IL4, IL8* and CSF2. Collectively, these data reveal specific porcine genes with differences in gene expression kinetics that may be responsible for the variation in disease progression observed in swine infected with *Typhimurium* compared to *Cholerae suis*.

The polymorphism of the sixth intron eleven sino-foreign in swine was reported that this polymorphism site was caused by CA-TG mutation at 278-279 base sites of the *Nramp1* gene, resulting in failure of reorganization of restriction enzyme sites by *Nde*I (Wu *et al.*, 2007). The restriction enzyme sites of *Nde*I tend to be the same in gene frequency and genotypic frequency of each breed of *NRAMP1* among different pig breeds. However, the correlation between gene polymorphism and disease resistance of *NRAMP1* as function gene was not being reported. It was reported that the porcine *SLC11A1* gene consist of 15 exons and 14 introns in pigs. All introns were sequenced and their nucleotide sequences were submitted to Gene Bank. The exon/intron boundaries were determined by comparing cDNA sequence with amplified genomic DNA sequences. Mutational analysis was performed on exonic and neighboring intronic region by denaturing high-performance liquid chromatography (DHPLC) and sequencing confirmation. Among the identified 40 polymorphisms, six were reported to be located in exons and 34 in introns. Two exonic polymorphisms are non-synonymous changes (D6H and V175I), three are synonymous changes (S23, G33 and I155), and one is in 3' UTR. They opined that the availability of the fine genomic organization and identification of the polymorphism facilitate the evaluation of the functional role of porcine *SlC11A1* genes to disease resistance or susceptibility.

Conclusion

Breeding for genetic resistance is one of the promising ways to control the infectious diseases. Increase in host resistance is the most important method for controlling such diseases, but no breed is totally resistant.

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The total resistance to these diseases is the ultimate goal and its progress towards achievement could be enhanced through introgression of resistance genes to breeds with low resistance. There is need to explore more and more SNPs in our population and establish its association with diseases but it is also expected that all SNP-based studies should be carefully crafted and well-designed from the beginning so that the utility of that particular study will exist at sent for some time. 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.

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