Original Research

Detection of Polymorphism in Coding Region of *SLC11A1* Gene in Jersey Crossbred Cattle

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Abstract

SLC11A1 gene (Solute Carrier 11 A1 gene), formerly called as Natural Resistance Associated Macrophage Protein 1 (NRAMP1) plays a critical role in innate immunity and mutation of this gene is responsible for susceptibility to a number of intracellular pathogens. The polymerase chain reaction - restricted fragment length polymorphism (PCR-RFLP) analysis (Pst I) of exon 11 in 52 Jersey crossbred cattle revealed presence of three genotypes CC (231bp), CG (231 bp, 200 bp, 31 bp) and GG (200 bp, 31 bp) confirming C > G transversion in coding region. The genotypic frequencies in pooled samples were estimated as 0.24 (CC), 0.48 (CG) and 0.28 (GG). This polymorphism observed in exon 11 is due to single nucleotide polymorphism (C > G). However, no polymorphic pattern was observed in exonic regions 4-5 and 5-6.

Key words: Jersey Crossbred Cattle, *NRAMP1*, SNP, *SLC11A1*, Transversion

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Introduction

The Nramp1 gene (coding for natural resistance-associated macrophage protein 1), previously known as Lsh/Ity/Bcg and recently renamed as *SLC11A1* (solute carrier family 11 member1), was first recognized in mice. The *SLC11A1* gene is associated with natural resistance against intracellular pathogens such as Mycobacterium *sp*., Salmonella *sp*., and Leishmania. It plays an important role in innate immunity by preventing bacterial growth in macrophages during the initial stages of infection (Paixa`o *et al*., 2007). In cattle, the *SLC11A1* gene sequence was first reported by Feng *et al*. (1996). Several studies reported possible associations of particular *SLC11A1* alleles with susceptibility to Johne's disease in cattle (Juste *et al*., 2005) and sheep (Reddacliff *et al*., 2005). *SLC11A1* gene has pleiotropic effects on macrophage function, that include increased chemokine KC, tumor necrosis factor-α, interleukin-1β, nitric oxide synthase and MHC class II expression having significant role in resistance to intracellular pathogens (Awomoyi, 2007).

Furthermore, an association between Crohn's disease, MAP infection with *SLC11A1* gene has been reported (Sechi *et al.,* 2006). Though vaccination helps to prevention and control of diseases in livestock, but it cannot eradicate the diseases. In these circumstances, marker assisted selection and breeding for improving disease resistance in animals can be effective strategy for the control of diseases. The goal of present study is to associate the presence of single nucleotide polymorphism (SNPs) in *SLC11A1* gene with Johne's disease suspected crossbred dairy cattle.

Materials and Methods

A total of 52 blood samples were collected (40 blood samples were JD suspected crossbred cattle and 12 normal crossbred cattle) from Jersey crossbred cattle. Animals were classed as suspected based on symptoms of JD like diarrhea, weakness, weight loss, etc. Genomic DNA was isolated from the venous blood using standard phenol chloroform extraction method by Sambrook *et al.* (1989). Primers P1, P2 and P3 (Table 1) were used to amplify the exon 4-5, 5-6 and 11 respectively of the *SLC11A1* gene as described by Zhang *et al.* (2009).

Table 1: Primer pairs for amplification of exon 11of bovine *SLC11A1* gene

Position	Primer Name	Sequence	PCR amplicon size	T_m	Restriction enzyme	PCR-RFLP Pattern
Exon 4-5	P1	5'–GCGACTGGCTGCCCGGCT-3'	1200 bp	67°C	Bgl II	645 bp and 555 bp
		5'–CTGTGCCAATGACTTCCTGC–3'			CFR13I (Sau96I)	580 bp, 540 bp and 80 bp
Exon 5-6	P2	5' –GCCCCGCATTCTCCTCTGG–3'	600 bp	60°C	TaqI,	561 bp, 27 bp and 12 bp
		5'-CCGTAGTTATCGAGGAAGAG–3'			RsaI	493 bp and 107 bp
					Ava II	344 and 256 bp
Exon 11	P3	5'–AAGGCAGCAAGACAGACAGG–3'	231 bp	69°C	Pst I	231bp, 200 bp and 31bp
		5'– CAGCCAGGAGACCCACG–3'			Hae III	140 bp, 72bp and 19bp

Polymerase chain reaction (PCR) was carried out in a final volume of 25 µl reaction mixture containing 12.5 µl of amplicon redeye master mix, 10 pM of each forward and reverse primer and 50 ng of Genomic template DNA. The thermal cycling profile for the reaction include initial denaturation at 95°C for 3 min followed by 35 cycles of denaturation at 95° C for 30 sec, annealing at 60 ° C - 69° C for 30 sec, extension at 72° C for 1 min and final extension at 72° C for 10min (Table 1). The amplified products were then resolved through 1.5 percent agarose gel electrophoresis and visualized in UV transilluminator (Bio Rad, USA) after staining with ethidium bromide. PCR amplicons were subjected to overnight digestion at 35^{0}C using appropriate restriction endonucleases and then inactivated at 60^{0}C to 80^{0}C for 20 min. PCR-RFLP pattern of exonic regions 4-5 (1200 bp) was studied using *CFR13*I and *Bgl*II restriction enzyme digestion,

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exonic regions 5-6 (600 bp) using *Taq* I, *Rsa*I and *Ava*II restriction enzyme digestion and exon 11 using *Pst* I and *Hae* III (Table 1). Inactivated PCR-RFLP products were subjected to 2 per cent gel electrophoresis and visualized in UV transilluminator (Bio Rad, USA).

Result and Discussion

Johne's disease was confirmed as negative in all the suspected animals based on PCR technique. Based on PCR-RFLP pattern at *SLC11A1* exon 4-5 (Bgl II and CFR13I) , 5-6 (Taq1 and Rsai) and 11 (*Hae* III) regions did not reveal any polymorphism and all the samples showed only monomorphic pattern (Fig. 1, 2 and 3).

Figure 1. PCR amplification and PCR-RFLP pattern of *SLC11A1* gene :Exon 4-5

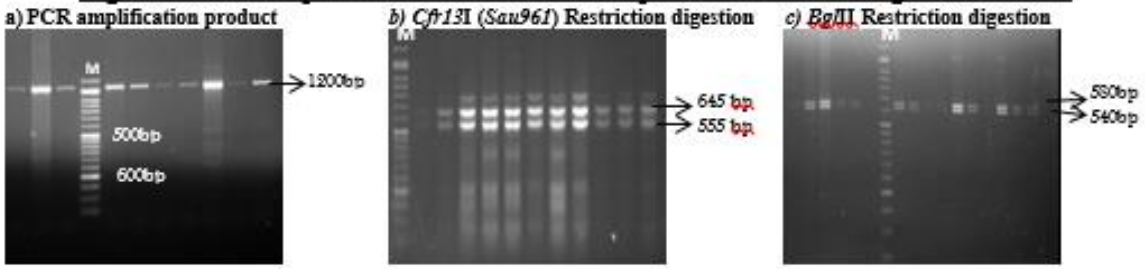


Figure 2. PCR amplification and PCR-RFLP pattern of *SLC11A1* gene :Exon 5-6 (600 bp)

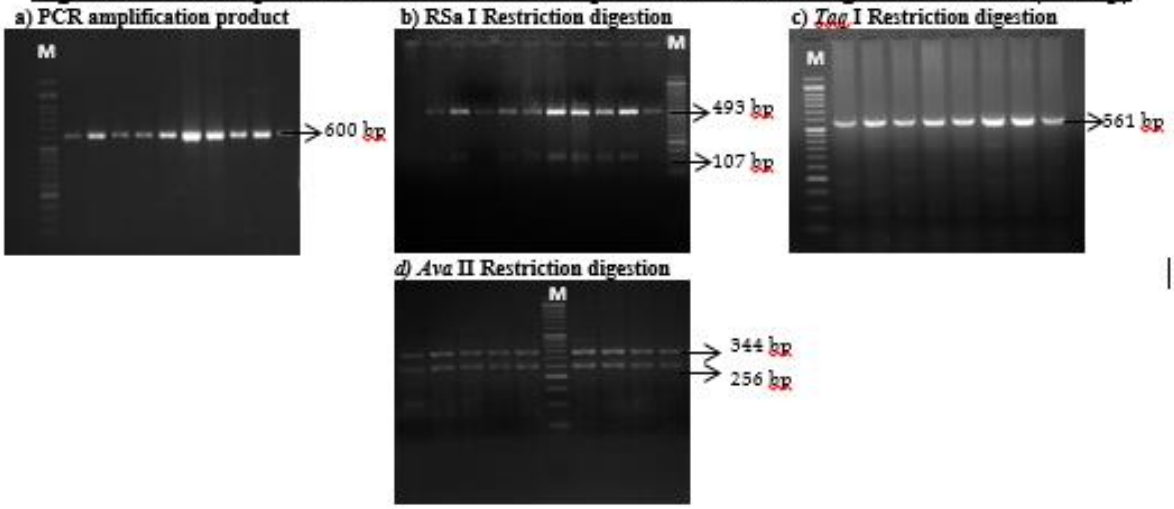
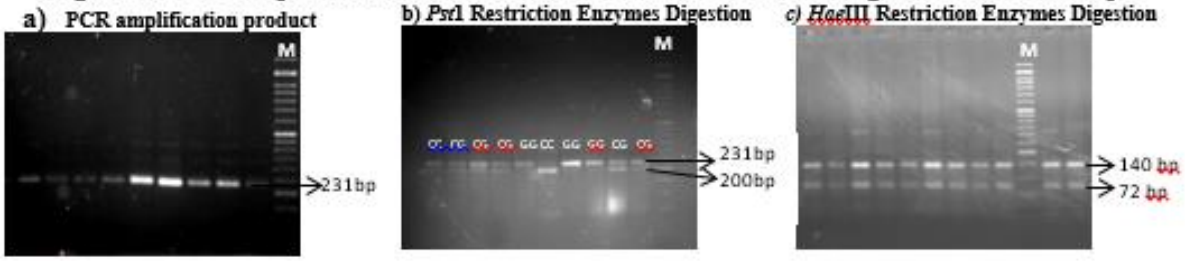


Figure 3. PCR Amplification and PCR-RFLP Pattern of *SLC11A1* gene: Exon 11 (231bp)



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However, PCR-RFLP analysis (*Pst* I) of Exon 11 revealed three genotypes CC (231 bp), CG (231 bp, 200 bp, 31 bp) and GG (200 bp, 31 bp) (Fig. 3b). This indicates the presence of SNP (confirming C > G transversion) in exon 11 coding region. Similar result was also identified at (g.1066 C > G) in exon 11 of Holstein by PCR-SSCP and sequencing (Zhang *et al*., 2009).

The genotypic frequencies in pooled samples were estimated as 0.24 (CC), 0.48 (CG) and 0.28 (GG) (Table 2). The frequency of C and G alleles were established as 0.58 and 0.42 respectively in normal healthy animals and it was as 0.47 and 0.53 respectively in suspected animals.

Table 2: Gene and genotype frequency of the alleles at exon 11 of *SLC11A1* gene in Jersey crossbred cattle

Samples (n)	Genotype	Genotype Frequency	Gene Frequency		χ^2 value
			C	G	(P>0.01)
Pooled (52)	CC	0.24	0.48	0.52	0.39
	CG	0.48			
	GG	0.28			
Normal (12)	CC	0.33	0.58	0.42	4.06
	CG	0.5			
	GG	0.17			
Suspected (40)	CC	0.23	0.47	0.53	
	CG	0.5			
	GG	0.27			

Chi-square test was carried out to test the null hypothesis for Hardy-Weinberg equilibrium. Results revealed no difference between observed and expected number (P =0.01). Fisher test shows no significant (df = 2, P > 0.01, χ^2 = 4.06) association between genotypic frequencies of healthy and suspected animals. However, the results reveal that C alleles were common in healthy animals with the frequency of 0.58 and G allele frequencies were common in suspected animals with frequency of 0.53. The presence of polymorphism in exon 11 is due to single nucleotide changes at g.1066 (C > G). The C > G transversion in coding region resulted in the amino acid change, which might affect the function of *SLC11A1* gene. *SLC11A1* may also have pleiotropic effect on the expression of MHC and surface antigen expression in mice (Lang *et al*., 1997).

Conclusion

In conclusion, polymorphism was not identified in exonic 4-5 and 5-6 regions. Though polymorphism in exon 11 of *SLC11A1* gene in cattle was observed, it failed to exhibit any association of the observed allelic variants with the resistance / susceptibility to Johne's disease as stated by Sadana *et al.* (2015). Further, studies need to be directed to explore polymorphisms throughout the entire *SLC11A1* gene in large number of samples to ascertain their suitability as potential genetic marker for Johne's disease resistance.

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