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Association of Single Nucleotide Polymorphisms in the DC-SIGN and SP110 Genes with Bovine Tuberculosis in Cattle

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SNPs in candidate genes for immune response DC-SIGN and SP110 have been reported for resistance /susceptibility to tuberculosis in humans as well as in animals. They are potential strong candidates for investigating genetic basis of disease resistance. Therefore the objective of this study was to explore the association of SNPs to susceptibility and resistance against TB infection in cattle. In case of SP110 gene both SNPs under study (rs41256732 and rs134537150) revealed polymorphism. Whereas in case of DC-SIGN gene two of SNPs (rs137338039 and s208436798) were found to be polymorphic while one SNP (rs134731570) revealed monomorphism. These findings point to the presence of these SNPs in our resource population. However, for the SNPs under investigation, the genotype as well as allele had nonsignificant effect on occurrence of bovine tuberculosis. Heterozygosity, PIC and allelic diversity values were estimated as low for rs208436798 while corresponding values for other SNPs were observed as moderate. Apart from rs134537150, the population was not in HWE for all the other the SNPs studied. In conclusion none of the SNPs studied were found to be associated but further validation in independent, large resource population and biological characterization are warranted with more effective case-control identification.

Key words: Polymorphism, Tuberculosis, SNP, Immune Response, Resistance.

Tuberculosis (TB) is a chronic bacterial disease in humans and animals caused by closely related acid-fast bacteria known as the Mycobacterium tuberculosis complex (MTBC)[1]. MTBC is composed of related bacterial sub-species including the typical human-associated pathogens M. tuberculosis and M. africanum, M. canettii and several lineages adapted to different mammal species that include M. bovis, M. microti, M. caprae, M. orygis, and M. pinnipedii[2]. In bovines TB is caused by M. bovis, which is an obligate aerobic, facultative intracellular parasite, usually of macrophages. Although cattle are considered to be the true hosts of M. bovis, the disease has been reported in many other domesticated and non-domesticated animals. It causes considerable difficulties on cattle dairy farms and poses health risks to the population that consumes products of animal origin. BTB has been reported in almost every country of the world but is endemic in Africa and Indian sub-continent[3]. 10-25 % loss in milk production efficiency has been reported in tuberculosis infected animals[4,5]. It costs an estimated $3 billion annually in global agricultural losses (Garnier) and is the fourth most important livestock disease worldwide[3]. A physiological characteristic of M. bovis infection is that it is an

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insidious, chronic process, which may take several weeks or months to become clinically patent. Thus cattle may become infectious long before they exhibit clinical signs or lesions. Consequently, the mainstay of TB control in cattle lies in the early detection and removal of M. bovis-infected animals. While traditional control measures such as vaccination, treatment and eradication exist, improving the genetic resistance to diseases among livestock populations is an effective alternate strategy as genetic gain is cumulative and permanent.

Genetic variation in susceptibility to tuberculosis has been observed in cattle. Higher resistance to TB has been reported among Bos indicus than Bos taurus[6,7]. Also, certain pedigree lines of cattle show greater and lesser susceptibility to the disease[8]. Estimates of the heritability of response to M. bovis PPD (purified protein derivative) in Irish herds were up 0.276[9], while heritability of TB susceptibility in British herds was estimated as 0.18 ± 0.04[10]. These encouraging findings indicate a role for genetics in a wider risk management strategy. Candidate genes, coding for proteins with very specific and unique roles in immune responses are potential strong candidates for investigating genetic basis of disease resistance. Nuclear body protein/ Intracellular pathogen resistance 1 (SP110/Ipr1) has been suggested to play an important role in preventing tuberculosis by mediating control of M tuberculosis within its prime target cell, the macrophage[11]. Polymorphisms of the human SP110 nuclear body protein gene, orthologous to murine Ipr1 have been reported to be associated with tuberculosis[12]. In an association study of 14 SNPs in two independent populations it was reported the SNP c.587A>G was significantly associated with Mycobacterium avium subspecies paratuberculosis (MAP) infection[13]. Dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN) has been shown to bind a variety of pathogens, including Mycobacterium bovis. In particular, C-type lectins detect pathogens by their characteristic carbohydrate structures and internalize them for further antigen processing and presentation[14]. DC-SIGN can initiate innate immunity by modulating toll-like receptors[15]. DC-SIGN together with other C-type lectins is involved in recognition of tumors by dendritic cells. Polymorphism of the promoter of DC-SIGN gene in Caucasians was studied at positions -336A/G and -871/G in 134 patients with pulmonary tuberculosis and 112 healthy individuals, who lived in the Irkutsk Region, Eastern Siberia. The bovine orthologue of human DC-specific ICAM-3 grabbing non-integrin C-type lectin (DC-SIGN) gene has recently been identified and functionally characterized. DC-SIGN receptor interacts and binds to M. bovis BCG It is worth contemplating that in cattle, as in humans, polymorphic variation in the DC-SIGN gene may be associated with TB resistance[16]. Therefore the objectives of this study were to explore polymorphism in candidate genes DC-SIGN and SP110 for susceptibility/resistance to tuberculosis and explore the association of polymorphism to susceptibility and resistance against TB infection in cattle.

MATERIALS AND METHODS

Experimental animals

Resource population under this investigation comprised of 245 cattle comprising Indigenous (Koshi, Sahiwal, Gir)/Nondescript and crossbred from Shri Mataji Gaushala, Barsana. All animals were kept in same herd had an equal opportunity of infection. Animals were screened for presence to bovine tuberculosis by Single Intradermal Tuberculin test (SITT) used to measure Delayed type hypersensitivity (DTH) response. An intradermal inoculation of 0.1 ml of tuberculin PPD antigen on neck region was carried out. The skin thickness was measured with vernier calipers before and 72 hours after inoculation. Based on thickness, cattle were classified into three groups : those showed marked swelling and skin thickness more than 4 mm (Positive), skin thickness < 4 mm and >2 mm (inconclusive) and no reaction > 2 mm (negative). The inconclusive animals were not included in the present investigation. A case and control resource panel of 35 positive and 45 negative animals was developed.

Sample collection and Isolation of Genomic DNA

About 6 ml of blood was collected from jugular vein in tubes containing EDTA and stored at -20°C. Genomic DNA was isolated from whole blood by using Promega Wizard ® Genomic DNA Purification Kit as per manufacturer's protocols.

The concentration of DNA was determined using Qubit Flurometer. DNA quality was also checked by running the sample in 1 percent agarose gel electrophoresis. One microliter of genomic DNA was resolved on 1% agarose gel stained with ethidium bromide or SYBR® Safe DNA gel stain and quantification was made by comparing the intensity of the band with the intensity of a known quantity of lambda DNA. Only thick DNA band and without smearing were chosen for further processing.

Genotyping of SNPs

Primers for the 5 SNPs in SP110 (rs41256732 and rs134537150) and DC-SIGN (rs137338039, rs134731570 and s208436798) genes were designed using Oligoanalyser for amplification of the loci. The detail of primers and restriction enzymes are being presented in Table 1. Respective amplicons were amplified under optimized polymerase chain reaction (PCR) conditions. The PCR product are resolved in 2.4% agarose gel and visualized under UV light after staining with ethidium bromide. The restriction enzyme digestion was made at the optimized conditions and the restriction digested products were resolved in 4% to 5% agarose gel and visualized under UV light after staining with ethidium bromide. Mass genotyping of all case-control resource population was done by using PCR-restriction fragment length polymorphism (PCR-RFLP).

Statistical analysis

The PROC ALLELE procedure of the SAS 9.3 used for the estimation of polymorphism information content (PIC), Hardy Weinberg Equilibrium (HWE) and heterozygosity. The PROC LOGISTIC procedure of SAS 9.3 software was also used to find association of allelic and genotypic frequencies with bovine TB.

RESULTS

The case-control population was genotyped by using PCR-RFLP for the two SNPs in SP110 and three SNPS in DC-SIGN genes. In case of SP110 gene both SNPs under study (rs41256732 and rs134537150) revealed polymorphism (Fig. 1 & 2). While in case of DC-SIGN gene two of SNPs (rs137338039 and s208436798) were found to be polymorphic while one SNP (rs134731570) revealed monomorphism (Fig. 3). These findings point to the presence of these SNPs in our resource population. Except for SNP A1415G (rs134537150), the population was not in HW equilibrium (HWE) for the SNPs studied.

Table 1. Details of single nucleotide polymorphisms (SNPs), primers and restriction enzymes (RE)

SNP I.D	Chromo-some no.	SNP	Primer Sequence (5'-3')	AT (°C)	RE	Fragments
rs41256732 (SP110)	2	C/T	TTCTGACTGCATCTGCCAAG TCCAGGAATCTGAGGTTTGG	57	Hpall	228, 190, 38
rsl34537150 (SP110)	2	A/G	GAATGTGAACGGTGGTGAGG CAACAGCTTCCAGGCTCATC	59	Earl	265, 193, 72
rsl37338039 (DC-SIGN)	5	C/T	AGGCTTAGAGAGTGA CTTGC ACCTTGAAAGCAGATTTGGC	57	Bsm Al	292, 197, 95
rsl34731570 (DC-SIGN)	5	C/T	ACCCCCAGAAATTCCTGAAC TAAATGCAGATTCCTGGGCC	58	BtsC I	319, 217, 102
rs208436798 (DC-SIGN)	5	C/T	CTTCTGGAAAAAAGGGGAGC TACTAAGGGGATGGGCATTC	57	Hpall	300, 201, 99

Table 2. Polymorphism at different SNPs and Chi-square probabilities for HWE

Locus	Alleles	Individuals	PIC	Heterozygosity	AllelicDiversity	P value forHWE
rs41256732 (SP110)	2	84	0.3749	0.5238	0.4997	0.6586
rsl34537150 (SP110)	2	84	0.3646	0.4881	0.4795	0.8698
rsl37338039(DC-SIGN)	2	84	0.2608	0.2857	0.3084	0.5004
rs208436798(DC-SIGN)	2	84	0.1494	0.0357	0.1626	<0.0001

Values for heterozygosity, PIC and allelic diversity were estimated as low for rs208436798 while corresponding values for other SNPs were observed as moderate (Table 2). The allelic and genotypic frequencies of the SNPs used for analysis are presented in Table 3 & 4. At SNP C912T (rs41256732) two alleles i.e. C and T and three genotypes i.e. CC, CT and TT were observed. While the CC genotype showed the fragments of 190 bp, and 38 bp, TT genotype showed the restriction fragment of 228 bp and the CT genotype showed the restriction fragments of 288 bp, 190 bp, 38 bp (Fig.1). While the frequency of T allele was 0.4857 in case and 0.4898 in control, C allele had the frequency of 0.5143 and 0.5102 in case and control population respectively. Similarly the frequency of genotype TT, CT and CC were 0.2000, 0.5714, and 0.2286 in case and 0.2449, 0.4898, and 0.2653 in control, respectively. The probability values showed that the genotype (P = 0.75) as well as allele (p =0.95) had nonsignificant effect on occurrence of bovine tuberculosis. The ODDs ratio of C verses T was 1.01(0.55-1.87; 95% CI), where as ODDs ratio of CC verses TT and CT verses TT were 1.05(0.29-3.80; 95% CI) ; 1.42(0.47-4.31; 95% CI) respectively (Table 3 & 4). At SNP A1415G (rs134537150) two alleles i.e. A and G and three genotypes i.e. AA, AG and GG were observed. While the GG genotype showed the fragments of 193 bp, and 72 bp, AA genotype showed the restriction fragment of 268 bp and the AG genotype showed the restriction fragments of 265 bp, 193 bp, 72 bp (Fig.2). While the frequency of G allele was 0.3286 in case and 0.4490 in control, A allele had the frequency of 0.6714 and 0.5510 in case and control population respectively. Similarly the frequency of genotype AA, AG and GG were 0.4000, 0.5429, and 0.0571 in case and 0.3265, 0.4490, and 0.2245 in control, respectively. The probability values showed that the genotype (P = 0.08) as well

Table 3. Allelic frequencies and their association with susceptibility to bTB in case-control population

SNP	Allele	Allele frequency		P value	Odds ratio (95% CI)
		Case	Control		
rs41256732 (SP110)	T	34(48.57)	48(48.98)	0.95	1.00
	C	36(51.43)	50(51.02)		1.01 (0.55-1.87)
rsl34537150 (SP110)	T	23(32.86)	44(44.90)	0.11	1.00
	C	47(67.14)	54(55.10)		1.66 (0.88 to 3.15)
rsl37338039(DC-SIGN)	G	51(72.86)	85(86.74)	0.12	1.00
	C	19(27.14)	13(13.26)		2.43(1.11 to 5.34)
rs208436798(DC-SIGN)	G	9(12.86)	6(6.12)	0.13	1.00
	A	61(87.14)	92(93.88)		0.44 (0.15 to 1.30)

Table 4. Genotypic frequencies and their association with susceptibility to bTB in case-control population

SNP	Genotype	Genotype frequency		P value	Odds ratio (95% CI)
		Case	Control		
rs41256732 (SP110)	TT	7 (20.00)	12 (24.49)	0.75	1.00
	CC	8 (22.86)	13 (26.53)		1.05 (0.29 to 3.80)
	CT	20 (57.14)	24 (48.98)		1.42 (0.47 to 4.31)
rsl34537150 (SP110)	GG	2(5.71)	11(22.45)	0.08	1.00
	AA	14(40.00)	16(32.65)		4.81 (0.90 to 25.52)
	AG	19(54.29)	22(44.90)		4.74 (0.93 to 24.16)
rsl37338039(DC-SIGN)	TT	19(54.29)	37(75.51)	0.09	1.00
	CC	13(37.14)	1(2.04)		5.84 (0.56 to 60.03)
	CT	16 (45.71)	11(22.45)		2.30 (0.86 to 6.10)
rs208436798(DC-SIGN)	TT	3(8.57)	3(6.12)	0.06	1.00
	CC	29(82.86)	46(93.89)		0.63 (0.11 to 3.33)
	CT	3(8.57)			>999.999 (<0.001 to >999.999)

as allele (p = 0.11) had nonsignificant effect on occurrence of bovine tuberculosis. The ODDs ratio of A verses G was 1.66(0.88-3.15; 95% CI), where as ODDs ratio of AA verses GG and AG verses GG were 4.81(0.90-25.52; 95% CI); 4.74(0.93-24.16; 95% CI) respectively (Table 3 & 4). At SNP C292T (rs137338039) two alleles i.e. C and T and three genotypes i.e. CC, CT and TT were observed. While the CC genotype showed the fragments of 197 bp, and 95 bp, TT genotype showed the restriction fragment of 292 bp and the CT genotype showed the restriction fragments of 292 bp, 197 bp, 95 bp (Fig.3). While the frequency of T allele was 0.7286 in case and 0.8674 in control, C allele had the frequency of 0.2714 and 0.1326 in case and control population respectively. Similarly the frequency of genotype TT, CT and CC were 0.5429, 0.3714, and 0.0857 in case and 0.7551, 0.2245, and 0.0204 in control, respectively. The probability values showed that the genotype (P = 0.09) as well as allele (p = 0.02) had nonsignificant effect on occurrence of bovine tuberculosis. The ODDs ratio of C verses T was 2.43(1.11-5.34; 95% CI), where as ODDs ratio of CC verses TT and CT verses TT were 5.84(0.56-60.03; 95% CI) ; 2.30(0.86-6.10; 95% CI) respectively.(Table 3 & 4). At SNP C300T (rs208436798 two alleles i.e. C and T and three genotypes i.e. CC, CT and TT were observed. While the CC genotype showed the fragments of 201 bp, and 99 bp, TT genotype showed the restriction fragment of 300 bp and the CT genotype showed the restriction fragments of 300 bp, 201 bp and 99 bp. While the frequency of T allele was 0.1286 in case and 0.0612 in control, C allele had the frequency of 0.8714 and 0.9388 in case and control population respectively. Similarly the frequency of genotype TT, CT and CC were 0.0857, 0.0857, and 0.8286 in case and 0.0612, 0, and 0.9389 in control, respectively. The probability values showed that the genotype (P = 0.05) as well as allele (p = 0.13) had nonsignificant effect on occurrence of bovine tuberculosis. The ODDs ratio of C verses T was 0.44(0.15-1.30; 95% CI), where as ODDs ratio of CC verses TT and CT verses TT were 0.63(0.11-3.33; 95% CI) and >999.999(<0.001->999.999; 95% CI) respectively (Table 3 & 4). At C319T (rs134731570) present in the exonic region of DC-SIGN gene, the mass PCR-RFLP revealed monomorphic pattern within as well as between the case and control population.

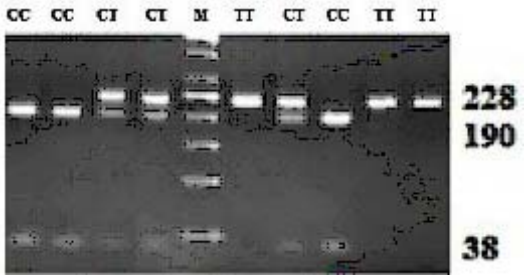

Fig.1. PCR RELP profile for SNP rs41256732 resolved at 5 % agarose gel, Lane M: 50 bp ladder

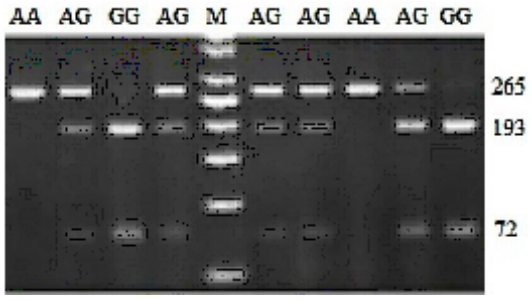

Fig. 2. PCR RELP profile for SNP rs134537150 resolved at 5 % agarose gel, Lane M: 50 bp ladder

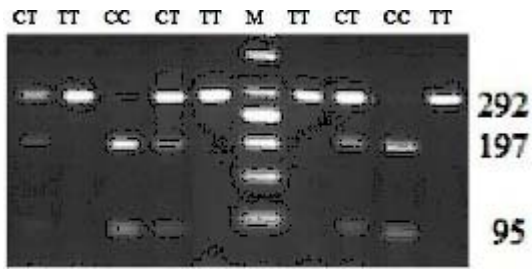

Fig. 3. PCR RELP profile for SNP rs137338039 resolved at 5 % agarose gel, Lane M: 50 bp ladder

DISCUSSION

Host genetic variation in disease resistance is mainly attributable to variability in host immune responses to infection[27]. Thus in the present study, an effort has been made to analyze the association of five SNPs in key genes involved in immune response against BTB between SITT positive and negative cattle. The two SNPs investigated in SP110 gene (rs41256732 and rs134537150) were located in the exonic and 3'UTR regions respectively. In present investigation, PCR-RFLP revealed polymorphism within as well as

between the case and control population. However for both the SNPs all the three genotypes obtained were not differing significantly (Pd"0.05) in case-control animals. Previously polymorphisms of the SP110 nuclear body protein (SP110) gene have been reported to be associated with tuberculosis (Tosh et al., 2006). Similar to our findings, association between SNP c.587A>G in SP110 gene and susceptibility to Mycobacterium bovis infection in cattle was studied and it was reported that neither SNP c.587A>G alleles nor genotypes showed significant association with susceptibility to Mycobacterium bovis infection[17]. The three SNPs studied for DC-SIGN gene (rs137338039, rs208436798 and rs134731570) lied in promoter, exonic and 3'UTR regions respectively. In the present study, Polymorphism was found at 2 SNPs (rs137338039 and s208436798) within as well as between the case and control population. However for both the SNPs all the genotypes obtained were not differing significantly (Pd"0.05) in case-control animals. Previously, comparison of DC-SIGN gene polymorphism in patients with tuberculosis and healthy controls revealed no significant differences in loci -336A/ G and -871A/G[18]. Previously it was tested whether polymorphisms in CD209, the gene encoding DC-SIGN, are associated with susceptibility to tuberculosis through sequencing and genotyping analyses in a South African cohort and observed an association between two CD209 promoter variants (-871G and -336A) and decreased risk of developing tuberculosis Barreiro et al., 2005. The linkage disequilibrium analysis revealed that the significantly linked loci/SNPs were rs41256732 with rs134537150; rs134537150 with rs208436798; rs137338039 with rs208436798 suggesting that there was linkage between alleles of these loci. In conclusion none of the SNPs studied were found to be associated but further validation in independent, large resource population and biological characterization are warranted with more effective case-control identification. In addition other SNPs of these concerned genes could be further exploited for association studies. After proper validation the associated SNPs can be incorporated in SNP panel for selection of animals with greater resistance to bovine tuberculosis.

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