SINGLE NUCLEOTIDE POLYMORPHISM IN CATTLE AND ITS ASSOCIATION WITH SUSCEPTIBILITY TO BOVINE TUBERCULOSIS

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ABSTRACT

Bovine tuberculosis is an important disease of cattle with zoonotic importance. A resource population comprising of 35 Case and 49 Control animals of indigenous cattle population was genotyped for a SNP from intergenic region of SLC6A6 gene, which have an established association with bovine tuberculosis susceptibility in Holstein-Freisian cattle population. Polymorphism in indigenous resource population at the targeted SNP revealed the presence of this SNP in indigenous cattle also. Both the alleles i.e. G and T and all possible three genotypes i.e. GG, GT and TT were observed in indigenous cattle. Low Polymorphic Information Content but high heterozygocity was observed at this SNP. The probability values showed that the genotype (P=0.38) as well as allele (P=0.53) had no significant effect on occurrence of bovine tuberculosis.

KEY WORDS: Bovine tuberculosis, indigenous cattle, SNP, SLC6A6

INTRODUCTION

Cattle is the major livestock species, constituting about 37.5% of the total livestock population and having a religious taboo attached, it shares the common habitat and common environment to a greater extent with the human, in comparison to other livestock species. Hence the concern for zoonotic diseases is more with cattle rearing. Tuberculosis (TB) is a major public health problem and the second most common cause of death from infectious disease in human. Among all developing nations, India is one of highest TB burden nation and having first place among 22 TB "High burden" countries. Genetic variation in susceptibility to tuberculosis has been observed in cattle. Early and recent studies indicated higher resistance to bTB among *Bos indicus* than *Bos taurus* (Garmichael *et al.*, 1941, Ameni *et al.* 2007*)*. Hence the recent focus is to identify the genetic polymorphism in the genes influencing immune response against the pathogens and to find its association with resistance/ susceptibility to the pathogen (Pant *et al*.,2011, Sun *et al*.,2012, Yuan *et al*.,2012). Another approach is genome wide scanning for identifying the DNA markers associated with resistance/ susceptibility to the pathogen. Finlay *et al.* (2012) conducted Genome Wide Association scan for bovine Tuberculosis susceptibility in Holstein-Friesian Dairy Cattle and identified a genetic region that may have a role in susceptibility to bovine tuberculosis. It contains three significant SNPs, located within a 65 kb region on BTA 22. While one SNP lied within an intron, two lied less than 60 kb upstream of the gene SLC6A6, the taurine transporter TauT. Hence in present study, Single Nucleotide polymorphism in intergenic region of SLC6A6 was studied in a Case: Control indigenous cattle resource population for its possible association with resistance/ susceptibility to bovine Tuberculosis.

MATERIALS AND METHODS

Source of Animal: Cattle population from Shri Mataji *Gaushala*, Barsana was selected for case and control study for bovine tuberculin trait in cattle. All animals were kept in same herd having equal opportunity of infection. A total of 245 animals were screened for single intradermal tuberculin test. Cattle of both sexes aged from 3-7 years were kept under similar management and feeding regime.

Screening of animals: Single intra-dermal tuberculin test was used for diagnosis of mycobacterium infection in herd which is globally one of the most accepted tests for diagnosis of bTB. Bovine Purified Protein derivative (PPD) 0.1 ml was injected in cervical region and measurement of skin taken before injecting PPD and after 72 hours of Bovine PPD injection. Animals were grouped into three groups i.e. with increase in skin thickness of $\geq$ 4 mm (positive), < 2 mm (negative) and of $\geq$ 2 to $\leq$ 4 mm (inconclusive). The inconclusive animals were not included in present investigation. A total of 35 tuberculin positive and 49 tuberculin negative animals were included in present study.

Blood collection and DNA isolation: Approximately 6 ml of venous blood from Jugular vein was collected in EDTA coating tube (2mg/ml of blood) and stored at -20°C till further use. DNA was extracted by using Promega Wizard® Genomic DNA Purification Kit as per recommended protocols.

PCR-RFLP assay

A QTL #20640 showing significant association with susceptibility to bovine tuberculosis encompassing a SNP i.e. rs42724727 at its peak on BTA22 was identified. The information regarding this SNP was retrieved from SNP database (www.ncbi.nlh.hih.gov/SNP). Suitable primers (Forward: 5'-AGG CAG TCC TGG ACC CCT C-3' and Reverse: 5'-GGT CTC CAT TTA GAT GCC ACC TCC TG-3') were designed to amplify a 319 bp region comprising the targeted SNP. PCR amplification conditions were optimized for amplification of the desired fragment. Twenty five µl of the PCR reaction mixture containing 10 pmole of each primers (forward and reverse), 1.5 mM $MgCl_2$, 1 X buffer, 200 µM of each dNTPs, 1 U Taq polymerase, 50 ng genomic DNA and Nuclease free water (NFW) to make up final volume was prepared. The cycling program used for amplification having following steps; initial denaturation (94°C for 4 min), followed by 35 cycles of 30 seconds denaturation at 94°C , 30 seconds of annealing at 63°C, 30 seconds of extension at 72°C and final extension of 5 min at 72°C. The amplified PCR products were resolved on 2.4 % agarose gel for their specificity. Restriction digestion was carried out in 25 µl reaction volume which included 20 µL of PCR product, 1.5 U of restriction enzyme (Apa*I*), 2.5 µl of 10X buffer and NFW to make volume up to 25 µl and incubated at recommended temperature as prescribed by manufacturer for 16 hours. The restriction enzyme treated PCR product, resolved on 4 % agarose.

Statistical analysis:

All the Case: Control resource population was genotyped on the basis of restriction enzyme digestion profile. The Genotypic frequencies of various identified genotypes were calculated by dividing the number of individuals of a particular genotype with the total Number of individuals of all genotypes (N). The allelic frequency was estimated as (2D+H)/2N, where D is number of homozygote animals of a particular allele, H is number of heterozygote animals having both alleles and N is the total number of individuals. The Heterozygosity, Polymorphic Information Content and Allelic diversity were estimated using PROC-Allele function of SAS Version 9.3. The association between various allelic variants with Bovine tolerance/susceptibility was worked out by suitable statistical techniques using different procedures of SAS 9.3. The PROC LOGISTIC procedure of SAS 9.3 was used to find association of allelic and genotypic frequencies with bTB. The ODDs ratio of genotypes was calculated in affected population versus their contemporary genotypes.

RESULTS AND DISCUSSION

The targeted SNP i.e. rs42724727 was identified in Holstein-Friesian cattle population. The polymorphic PCR-RFLP pattern for this SNP in our indigenous resource population revealed the presence of this SNP in Indian cattle population. All the three genotypes i.e. GG, GT and TT were observed. While the GG genotype showed the restriction fragments of 39 bp, 280 bp, GT genotype showed the restriction fragments of 319 bp, 39 bp , 280 bp and TT genotype showed the restriction fragments of 319 bp (Fig 1). The genotypic frequencies of GG, GT and TT genotypes were 0.18, 0.78 and 0.04, respectively. The overall allelic frequency of G and T allele were 0.57 and 0.43,

respectively. The polymorphic Information Content, heterozygocity and allelic diversity for the SNP were 0.3698, 0.7857 and 0.4898, respectively. The chi square test revealed that the population was not in HWE.

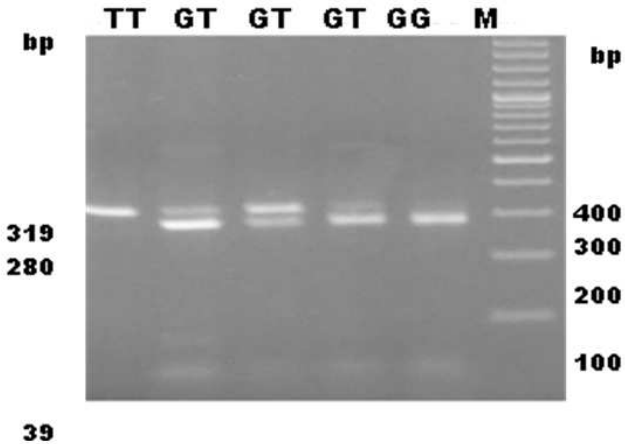
The allelic and genotypic frequencies of different alleles and genotypes in Case and Control individuals have been presented in Table 1. The frequency of G allele was 0.543 in case and 0.5918 in control whereas T allele had frequency of 0.457 and 0.401 in case and control population respectively. Similarly the frequency of genotype GG, GT and TT were 0.114, 0.857 and 0.029 in case and 0.225, 0.735 and 0.041 respectively in control. The probability values showed that the genotype (P=0.38) as well as allele (P=0.53) had no significant effect on occurrence of bovine tuberculosis. The ODDs ratio of G versus T 0.82 (0.44-1.52; 95% CI) and of GG verses TT (0.05 - 10.39: 95% CI) and GT versuse TT (0.73 and 1.67 (0.14 - 19.29; 95% CI) also revealed no significant differences between Case and Control populations.

These results suggested that though the polymorphism was observed in our Case: Control population for this SNP from intergenic region of SLC6A6, but neither the alleles or genotypes at this loci showed any significant effect on susceptibility to tuberculosis in cattle. However, Finlay *et al.* (2012) identified a genomic region on BTA 22, suggestively associated with tuberculosis susceptibility and it contains the taurine transporter gene SLC6A6, or TauT, which is known to have function in the immune system.

Table1: Allelic and genotypic frequency distribution at intergenic SNP (rs42724727) and their association with risk to bovine Tuberculosis

Alleles/ Genotypes	Allele	Frequency			p-Value	Odds ratio (95% CI)
		Case	Control	overall		
Alleles	G	38 (54.28)	58 (59.18)	96 (0.57)	0.53	0.82 (0.44 - 1.52)
	T	32 (45.72)	40 (40.12)	72 (0.43)		1
Genotype	G/G	4 (0.114)	11 (0.225)	15 (0.18)	0.38	0.73 (0.05 - 10.39)
	G/T	30 (0.857)	36 (0.735)	66 (0.78)		1.67 (0.14 - 19.29)
	T/T	1 (0.029)	2 (0.041)	3 (0.04)		1

Figure 1. Representative restriction enzyme digestion profile of Case: Control resource population for SNP rs42724727



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