POLYMORPHISM IN IFNGR2 GENE AND ITS ASSOCIATION WITH SUSCEPTIBILITY TO TUBERCULOSIS IN CATTLE

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ABSTRACT

A total of 245 indigenous cattle were screened for presence of bovine tuberculosis by using Single Intradermal tuberculin test. A resource population (35 Case and 49 Control animals) was developed. Polymorphism at one non-synonymous Single nucleotide polymorphism (SNP) locus from exonic region of IFNGR2 gene i.e. IFNGR2-C277T was studied by using Polymoerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). Polymorphic PCR-RFLP pattern with Hpa*II* in the resource population showed the existence of the SNP site in indigenous cattle also. Low level of polymorphism as well as heterozygocity was observed at this SNP. The frequency of C allele was 0.686 in case and 0.694 in control whereas T allele had frequency of 0.314 and 0.306 in case and control population respectively. Similarly the frequency of genotype CC, TC and TT were 0.486, 0.400, and 0.114 in case and 0.531, 0.326, and 0.143 respectively in control. The probability values showed that the genotype (P = 0.77) as well as allele (P = 0.91) had non significant effect on occurrence of bovine tuberculosis.

KEYWORDS: Tuberculosis, Cattle, IFNGR2, genetic polymorphism

INTRODUCTION

Bovine tuberculosis (bTB) is a chronic debilitating infectious disease caused by Mycobacterium bovis (*M. bovis*) that affects cattle as well as other domesticated animals (Moda et al., 1996). The disease is contagious and spread by contact with infected domestic and wild animals. Further, the *M. bovis* is a zoonotic organism and should be treated as a risk/hazard group III organism with appropriate precautions to prevent occurring infection in human. Till date, there is no effective treatment for treating tuberculosis infected animals. Many countries have test and slaughter policy to control tuberculosis but it is difficult to apply this policy in India due to religious taboos. Development of disease resistance markers and breeding can be helpful to fight against tuberculosis and control, especially in country like India where animals and human share common habitat and environment. Interferon-γ (IFN-γ) has a key role in T helper 1 (Th1)-type immune response against mycobacterial diseases (Lin and Flynn 2010). The IFNGR2 chains in combination with IFNGR1 chains make functional IFN-gamma receptor (IFNGR), which is associated with signaling machinery. IFNGR2 is generally the limiting factor in IFN-gamma responsiveness, as the IFNGR1 chain is usually in surplus (Bernabei et al., 2001). Mutations in IFNGR2 are a cause of autosomal recessive mendelian susceptibility to mycobacterial disease (MSMD), also known as familial disseminated atypical mycobacterial infection (Al-Muhsen et al., 2008; Zhang et al. 2008) in human being. There is no report of association of IFNGR2 mutation with the susceptibility or resistance to bovine tuberculosis in cattle. Therefore, an attempt has been made to study the genetic polymorphism in IFNGR2 gene in cattle and to find an association of the polymorphism in IFNGR2 with the resistance or susceptibility to bovine tuberculosis (bTB) in Indian cattle.

MATERIALS AND METHODS

Case: Control Resource population: A Cattle population from Shri Mataji *Gaushala*, Barsana, Uttar Pradesh was used for developing Case: Control resource population. All animals were kept

under similar environment, hence had equal opportunity of infection. A total of 245 cattle between 3 to 7 years of age were tested for bovine tuberculosis using Intra-dermal Tuberculin test, which is based on delayed type hypersensitivity reaction in response to injection of 0.1 ml of Bovine Purified Protein derivative (PPD) in cervical region. Simultaneously, 0.1 ml of Phosphate Buffer Saline (PBS) was also injected in the cervical region at another site, approximately 10-15 cm apart from PPD injection site. Skin thickness was taken before the injection as well as after 72 hours of injection using Vernier Calliper. Difference in skin thickness (D) after 72 h was measured as $(T_{72}-T_0) - (C_{72}-C_0)$, where T_0 and T_{72} are the skin thickness before and after 72 h of PPD injection, where C_0 and C_{72} are the skin thickness before and after 72 h of equal volume of PBS. Animals with the value of "D" more than 4 mm, were taken as TB positive (Case), whereas those having "D" value of less than 2 mm, were taken as TB negative (Control). The animals having "D" value between 2 to 4 were considered as inconclusive, hence not included in the Case: Control resource population.

Blood collection and DNA isolation: About 6 ml of whole blood was collected from Jugular vein in a tube containing EDTA (@ 2mg/ml of blood). Blood samples from all the 84 animals were taken and stored at -20°C till further use. The genomic DNA was extracted by using Promega Wizard® Genomic DNA Purification Kit as per recommended protocols. The quality of the genomic DNA was assessed through resolving the extracted DNA on 1 % agarose gel. The quantity of DNA was estimated by using Qubit® 2.0 Flurometer (Invitrogen, USA).All the samples were diluted to a final concentration of about 50 nmole per µl.

PCR-RFLP assay : The Single Nucleotide Polymorphism (SNP) locus i.e. rs134539971 in IFNGR2 was selected from the database (http://www.ncbi.nlm.nih.gov/snp) on the basis of its assay ability through PCR-RFLP (Table 1). Suitable forward primer (5' TTC GAA CCA AGG TGC TGT TG-3') and reverse primer (5'- CCC TTA AGT GAG AGC AGT GTG-3') were designed for amplification of the IFNGR2 gene region encompassing the targeted SNP. The 25 µl PCR reaction was comprised of 1 µl genomic DNA, 5 µl of 5X PCR buffer, 0.2 µl dNTPs, 1.5 µl of 25 mM $MgCl_2$, 1 µl of each primers (10 pMole/ µl), 1 unit Taq polymerase and Nuclease free water (NFW) to make total volume to 25 µl. The amplification profile was comprised of: initial denaturation at 94°C for 4 min, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 58.5°C and 30 seconds at 72°C; and final extension of 5 min at 72°C. Amplified PCR products were tested on 2.4 % agarose gel for their specificity. The Restriction enzyme digestion reactions were comprised of 20 µl of PCR product, 1.5 U of restriction enzyme (Hpa*II*), 2.5 µl of 10X buffer and nuclease free water 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 gel. The individuals were genotyped on the basis of restriction enzyme pattern exhibited by each individual.

Statistical Analysis: All the individuals of Case: Control resource population was genotyped on

Table 1 details of SMP

SNP	Nucleotide change	Type	Amino Acid Change	5'-3' Primer Sequence	Restriction Enzyme	Amplicon Size (bp)	RE digestion profile (in bp)		
							CC	CT	TT
IFNGR2-C277T	C by T at 277 b position	Non-synonymous	Arg by Gly	TTC GAA CCA AGG TGC TGT TGCCC TTA AGT GAG AGC AGT GTG	HpaII	334	128, 206	128, 206, 334	334

the basis of restriction enzyme digestion profile. The Genotypic and allelic frequencies were estimated as follows

Genotypic Frequency = D_1 or D_2 or H / N,

Allelic frequency = (($2D_1$ or $2D_2$)+ H)/2N

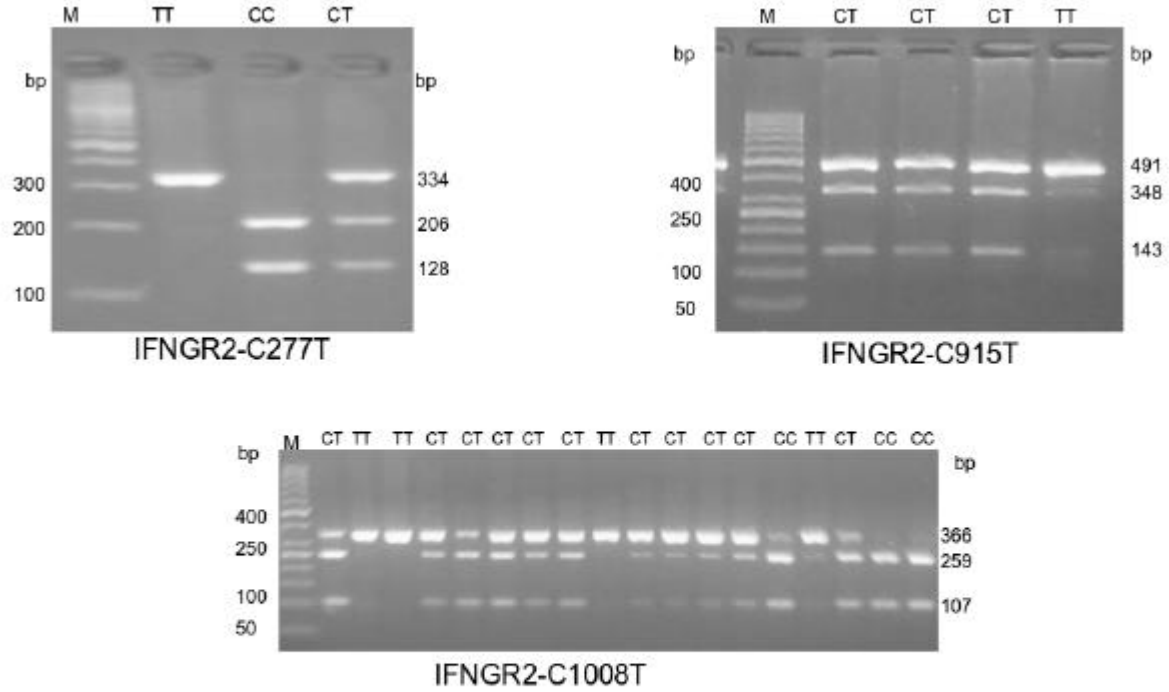
Where, D_1 is numbers of homozygote animals for first allele, D_2 is number of individuals for second allele, H is numbers of heterozygote animals and N is the total number of individuals.

PROC-Allele function of SAS Version 9.3 was used to estimate Heterozygosity, Polymorphic Information Content and Allelic diversity. PROC LOGISTIC function 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 (SAS Inc, 2012).

RESULTS AND DISCUSION

The designed primers amplified the 334 bp region of IFNGR2 gene, encompassing the targeted single nucleotide polymorphism locus SNP i.e. rs134539971(Fig1). At this SNP, replacement of C with T have abolished the restriction site of *HpaII* restriction enzyme, hence on the restriction

Figure 1. Representative restriction enzyme digestion profile of Case: Control resource population for SNP rs134539971



Representative genotyping profile of IFNGR2 gene at loci +277C/T, +915C/T, and +1008C/T. Genotype written at upper rows and M means 50 and 100 bp DNA ladder.

digestion of the 334 bp fragment with *HpaII*, the C/C genotypes exhibited two restriction fragments of 206 bp and 128 bp, whereas T/T genotypes showed only one undigested band of 334 bp. The C/T genotypes showed three bands i.e. 334 bp, 296 bp and 128 bp. Since this SNP was taken from the data base and was from *Bos Taurus*, existence of this SNP in our indigenous cattle population *(Bos Indicus)* was revealed from the polymorphic restriction enzyme digestion profile. In our resource population, all the three genotypes i.e. C/C, T/C and T/T were observed (Figure 1) and had the frequency of 0.51, 0.36 and 0.13, respectively. The allelic frequency of C allele and T allele was 0.69 and 0.31, respectively. Low estimate of Polymorphic Information Content (0.3361),

heterozygocity (0.3571) and allelic diversity (0.4274) revealed low polymorphism as well as low heterozygocity at the SNP site. The chi square test revealed that the population was not in Hardy Weinberg Equilibrium.

Table 2: Allelic and genotypic frequency at IGNGR2-C277T SNP and its association with susceptibility/ resistance to bovine tuberculosis

Alleles/ Genotypes	Allele	Allele / Genotypic frequency			P-Value	Odds ratio (95% CI)
		Case	Control	Total		
Alleles	C	0.686	0.694	0.69	0.91	0.96 (0.49-1.86)
	T	0.314	0.306	0.31		1
Genotypes	C/C	0.486	0.530	0.51	0.77	1.44 (0.29 – 4.51)
	C/T	0.400	0.327	0.36		1.53 (0.37 -6.35)
	T/T	0.114	0.143	0.13		1

The allelic and genotypic frequencies of different alleles and genotypes in Case and Control individuals have been presented in Table 2. While The frequency of C allele was 0.686 in case and 0.694 in control whereas T allele had frequency of 0.314 and 0.306 in case and control population respectively. Similarly the frequency of genotype C/C, T/C and T/T were 0.486, 0.400, and 0.114 and 0.531, 0.326, and 0.143 in case and control respectively. The probability values showed that the genotype (P = 0.77) as well as allele (P = 0.91) had not significant effect on occurrence of bovine tuberculosis. The ODDs ratio of C verses T was 0.96 (0.49-1.86; 95% CI), where as ODDs ratio of CC verses TT and TC verses TT were 1.44 (0.29 – 4.51; 95% CI) and 1.53 (0.37 -6.35; 95% CI) respectively. There is no report of any association study between SNPs from IFNGR2 with the susceptibility to bovine TB, however, there are reports in human that mutation in IFNGR2 causes impaired immune against mycobacterium tuberculosis (Newport *et al*., 1996). More recently, Hijjakata *et al*. (2012) found an association of one IFNGR2 3'UTR SNP i.e. rs1059293 with susceptibility to TB in human. The SNP i.e. IFNGR2-C227T was from exonic region of IFNGR2 gene and was non-synonymous also i.e. resulting a change of Arginine to Glycine, but present results suggested no association of this SNP with susceptibility or resistance to bovine tuberculosis.

REFERENCES :

Al-Muhsen, S. and Casanova J.L, (2008). J Allergy Clin Immunol. 122 **(6)**: 1043–51.

Bernabei, P., Coccia, E. M., Rigamonti, L., Bosticardo, M., Forni, G., Pestka, S., Krause, C. D., Battistini, A., Novelli, F. (2001). J. Leukoc. Biol. **70**: 950 –960.

Hijikata, M., Shojima, J., Matsushita, I., Tokunaga, K., Ohashi, J., Hang, N. T. L. Horie, T., Sakurada, S., Hoang, N. P., Thuong , P. H., Lien, L.T. and Keicho, N. (2012). Hum Genet **131**:675–682

Lin, P. L., and Flynn, J,L. (2010). J. Immunol.**185**: 15–22.

Moda, G., Daborn, C. J., Grange, J.M. and Cosivi, O. C. (1996). Tubercle and Lung Dis. **77**: 103-108.

Newport, M. J., Huxley, C. M., Huston, S., Hawrylowicz, C. M., Oostra, B. A., Williamson, R. and Levin, M. (1996). N Engl J Med. **335**:1941–1949.

SAS Institute Inc. 2012. What's New in SAS® 9.3. Cary, NC: SAS Institute Inc.

Zhang, S.Y., Boisson-Dupuis, S., Chapgier, A., Yang, K., Bustamante, J., Puel, A., Picard, C., Abel, L., Jouanguy, E. and Casanova, J.L. (2008). Immunol Rev. **226**:29–40.

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