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Influence of non-MHC genes on lymphocyte response to *Mycobacterium tuberculosis* antigens & tuberculin reactive status in pulmonary tuberculosis

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Background & objectives **: Major histocompatibility complex (MHC) genes are known to influence the immune functions. In the present study, the influence of non-MHC genes such as mannose binding protein (MBP), vitamin D receptor (VDR) and interleukin-1 receptor antagonist (IL-IRA) gene polymorphisms on lymphocyte response to** *Mycobacterium tuberculosis* **culture filtrate antigen (10 µg/ml) was studied in 44 patients with active pulmonary TB and the family contacts (35) and in 32 normal healthy subjects. The influence of these gene polymorphisms on tuberculin (1TU of PPD of** *M. tuberculosis)* **reactivity status in 146 pulmonary TB patients was also studied.**

Methods **: The MBP and VDR genes were amplified using polymerase chain reaction (PCR) and genotyping was carried out using sequence specific oligonucleotide probes by dot blot and IL-1RA by agarose gel electrophoresis.**

Results **: A significantly decreased lymphocyte response to** ***M. tuberculosis*** **antigen was seen in pulmonary TB patients positive for functional mutant homozygotes of MBP (00) compared to heterozygote carriers (AO; P<0.02) and wild homozygotes (AA; P<0.01). The variant mutant genotype (tt) of VDR gene was associated with an increased lymphocyte response in control subjects compared to active TB patients with tt genotype (P<0.05). Heterozygote carriers of MBP (AO) were associated with a significantly (P<0.001) decreased tuberculin reactivity compared to wild homozygotes (AA). The VDR genotype Tt (heterozygote carrier) was associated with an increased tuberculin reactivity in female TB patients as compared to male patients (P<0.001).**

Interpretation & conclusions **: The present study suggested that MBP and VDR genes influence the cell mediated immune response in pulmonary TB patients. Non-MHC genes along with HLA-Class II genes/gene products may be playing an immunoregulatory role in the mechanism of susceptibility/resistance to tuberculosis.**

Key words Gene polymorphisms - IL- 1RA - lymphocyte response - MBP - *Mycobacterium tuberculosis* - pulmonary-TB tuberculin reaction - VDR

The progression of tuberculosis is regulated/ controlled almost entirely by the cell mediated immune response (CMI) of the host against the pathogen. -The immune response against *Mycobacterium tuberculosis* involves both macrophages and lymphocytes[1], the macrophages being the major cells involved in the CMI response. The uptake of pathogens by the macrophages is

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facilitated by various serum proteins and receptors of macrophages such as mannose binding protein (also known as mannose binding lectin), complement components, mannosyl receptors, complement receptors and Fc receptor for IgG antibody[2,3]. Vitamin D3 (1,25 dihydroxy vitamin D3) is an immunoregulatory hormone and activates monocytes/macrophages and stimulates cell mediated immunity[4]. Vitamin D is one of the few mediators shown to impair the growth of *M. tuberculosis* in the macrophages[5]. The effects of vitamin D are exerted by the interaction through vitamin D receptor. It has been suggested that mutant alleles of the VDR gene region may be associated with increased or decreased VDR mRNA expression[6].

Monocytes/macrophages play a major role in the host defense against *M. tuberculosis* and secrete inflammatory cytokines at the site of infection. IL-1 receptor antagonist (IL- 1RA) is one of the cytokines which competes for the IL-l binding site. IL- la and IL-1ß exert their effects by interaction with IL-l receptor and the effect is antagonized by IL-1RA[7]. Macrophages from heterozygote carriers of IL-1RA allele 2 have been shown to produce more IL-1RA and less IL-la than other genotypes[8]. MBP, VDR and IL-IRA genes play a role on macrophage activation as well as production of cytokine factors. The cytokines released by the macrophages and lymphocytes are involved in eliciting a good CMI response at the site of infection. Our recent study revealed that functional mutant homozygotes (FMH) of MBP is associated with the susceptibility to pulmonary TB[9]. Further, variant mutant genotype (tt) of VDR is associated with the susceptibility to pulmonary TB in female patients[10].

Earlier studies revealed that HLA-Class II genes/ gene products are associated with genetically controlled differences in delayed hypersensitivity skin responses to *M. leprae* [11] and *M. tuberculosis* [12-14]. Since, non-MHC genes/gene products also regulate the immune function, the goal of the present study was to find out whether the gene variants of non-MHC genes such as MBP, VDR, IL- 1RA influence the lymphocyte response to *M. tuberculosis* culture filtrate antigen and tuberculin reactivity status to purified protein derivative (PPD) of *M. tuberculosis* in pulmonary TB patients.

Material & Methods

Study subjects :

(i) Active tuberculosis patients (ATB) – Patients attending the Tuberculosis Research Centre (TRC), Chennai, with respiratory symptoms and radiographic abnormalities suggestive of pulmonary TB were studied. These patients had sputum positive for *M. tuberculosis* by smear and culture. Blood samples were taken before the start of chemotherapy. A total of 44 patients aged 37.1 ± 1.7 yr (mean ± SE) were studied.

(ii) Controls – Control subjects comprised 32 normal healthy subjects (NHS) and 35 family contacts (CT); spouses of patients of pulmonary TB and TB spine. Normal healthy subjects were from among the staff of TRC. The family contacts lived with the patients before, during and after treatment for a period of 10-15 yr. All the healthy subjects and the family contacts (spouses) were clinically normal at the time of blood sample collection. The patients and spouses were not consanguineous to each other. The patients and the controls were randomly selected and matched for ethnic group. They were Tamil speaking south Indian population (belonging to different communities/castes) living in and around Chennai, Tamil Nadu, India. The mean age with SE for the family contacts was 38.8 ± 2.0 and for the normal healthy subjects it was 38.5 ± 1.5 yr.

M. tuberculosis culture filtrate antigen : H37Rv strain of *M. tuberculosis* was grown for 6 wk (late logarithmic phase) as a surface pellicle on Sauton's medium at 37°C. The culture was centrifuged at 3,000 rpm for half an hour to remove the bacilli and the supernatant was filtered through a Seitz filter (0.45 pm) (Seitz-Filter-Werke GmbH und Co., Badkreuznack, Germany) to ensure complete removal of the bacilli. The culture filtrate antigens were precipitated with 80 per cent ammonium sulphate and dissolved in sterile phosphate buffered saline (PBS; pH 7.4). The excess salt was removed by dialysis using 0.2 *M,* 0.1 *M* and. 0.02 *M* PBS (PH 7.4) at 4°C. The dialysed protein was lyophilised and resuspended in minimal volume of sterile PBS. The protein concentration was estimated by Lowry's procedure[15] against bovine serum albumin as

standard. The culture filtrate protein antigens were aliquoted and stored at -20°C until required. The antigen concentration used for lymphocyte response was 10.0 µg/ml of culture.

Lymphocyte transformation test: Ficoll-Hypaque separated peripheral blood mononuclear cells (PBMC) of pulmonary TB patients, patient contacts and normal healthy subjects (NHS) were suspended in RPMI tissue culture medium (Sigma Chemical Co., St. Louis, USA) supplemented with 2mM L-glutamine (Flow Laboratories, Scotland), gentamycin (10 µg/ml; M.A. Bio Products, Walkersville, Maryland, USA), fungizone (5 µg/ml; Squibb, Princeton, NJ, USA) and 10 per cent autologous plasma for control subjects. Autologous serum was used instead of plasma in the cultures set up with samples from pulmonary tuberculosis patients to avoid clotting in the culture. No difference was observed in the counts when autologous plasma or serum was used. 0.1 x 10^6 PBMC in 200 µl were cultured with *M. tuberculosis* culture filtrate antigen at 10 µg/ml concentration. Cultures without any stimulation served as controls. Cultures with *M. tuberculosis* culture filtrate antigen were kept for 144 h (6 days) in a 96 well 'U' bottom tissue culture plate (Costar, Cambridge, MA, USA), at 37°C and 5 per cent carbon dioxide in a CO_2 incubator (Flow Laboratories Ltd., Rickmansworth, Hertz, UK). The lymphocyte cultures were set up at least in triplicate. Tritiated thymidine (^3H; BARC, Mumbai, India) was added to each well (1 µCi/well) 16 h before termination. The cells were harvested onto glass fibre filters using a PHD cell harvester (Cambridge Technology, Inc. Water Town, MA, USA). The lymphocyte response to *M. tuberculosis antigen* was measured by tritium labelled thymidine uptake by the cells in a scintillation system using a Beta counter (LKB, Wallac, Oy, Turku, Finland) and expressed as counts per minute (cpm). The mean cpm value of the triplicate culture was calculated and the stimulation index (SI) was derived using the formula:

$$SI : \frac{\text{mean cpm of antigen stimulated cell cultures}}{\text{mean cpm of cell cultures without antigen}}$$

Tuberculin skin test: The results on tuberculin reactive status of 146 pulmonary TB patients of controlled clinical trials was analysed retrospectively to find out whether the gene variants of non-MHC genes influence the tuberculin status.

DNA extraction: DNA was extracted from the peripheral blood white cells using salting out procedure[16].

Genotyping of candidate genes: The genotyping of mannose binding protein (MBP) gene, vitamin D receptor (VDR) gene and Interleukin-1 receptor antagonist (IL-IRA) gene of pulmonary tuberculosis patients has been described elsewhere[9,10]. The work on lymphocyte transformation and the genotyping of MBP and VDR were carried out during 1995 to 1997.

Statistical analysis: Frequencies of the MBP, VDR and IL-1RA candidate genotypes in the pulmonary TB patient groups and controls were analysed using χ^2 analysis employing the Statcalc program (Epi Info, Version-5; USD; Stone Mountain, GA, USA). The results on the lymphocyte response and tuberculin reactivity are expressed as mean ± standard error (SE). Statistical significance was assessed using Kruskal-Wallis one-way analysis of variance by ranks and in case of a significant result, multiple comparison test was carried out to identify the pairs of study groups having significantly different levels of the parameter.

Results

A significantly decreased lymphocyte response to *M. tuberculosis* culture filtrate antigen was seen in active TB patients positive for functional mutant homozygotes (FMH) (00) of MBP as compared to heterozygote carriers (AO) and patients homozygous for wild type alleles (AA). (MBP: AA vs OO $P<0.01$; AO vs OO ($P<0.02$; Fig. 1). No dramatic difference in the lymphocyte response to *M. tuberculosis* antigen was seen with the vitamin D receptor genotypes TT (wild type homozygotes) and Tt (heterozygote carrier) of normal healthy subjects, contacts and active pulmonary tuberculosis patients. However, a significantly increased lymphocyte response was observed in normal healthy subjects and patient contacts compared to active TB patients with mutant genotype (tt) of VDR (NHS vs ATB $P<0.02$; CT vs ATB $P<0.05$). No difference in the

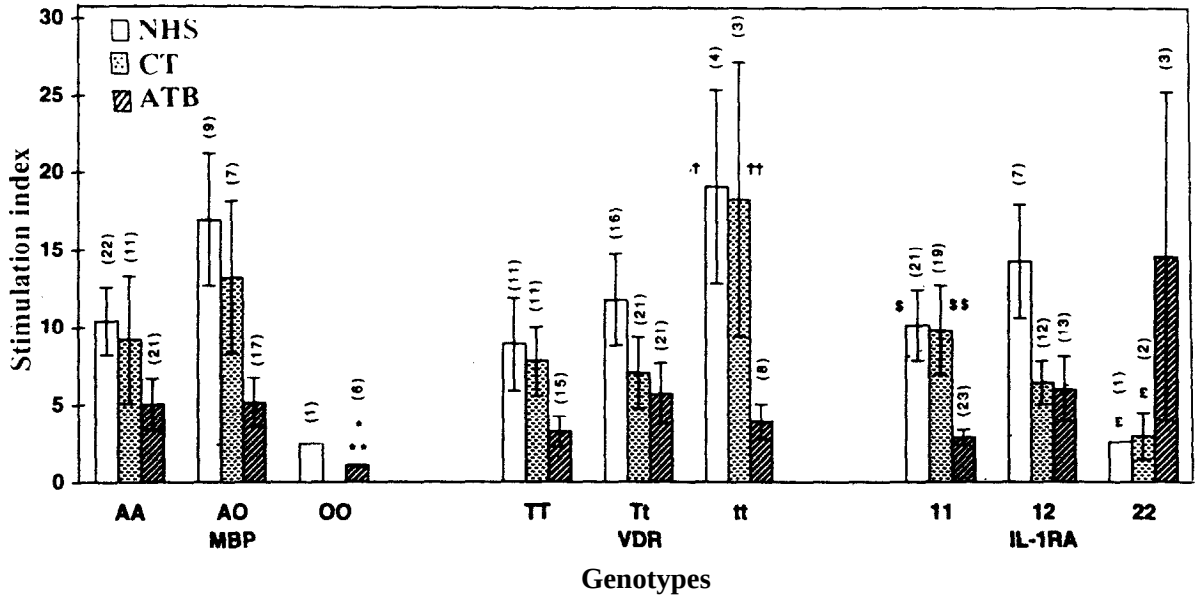

Fig. 1. influence of variant genotypes of MBP, VDR and IL-1RA genes on *M. tuberculosis* culture filtrate antigen induced lymphocyte response in active pulmonary TB (ATB) patients, family contacts (CT) and normal healthy subjects (NHS). Results are mean ± SE. Numbers in parantheses represent the number of subjects studied in each group.

Mannose binding protein (MBP) genes: A, wild type allele; O, mutant alleles of MBP genes 52, 54, 57; AA, wild type homozygotes: AO, heterozygote carriers; OO, functional mutant homozygotes; ATB *$P<$ 0.01 compared to AA; **P <0.02 compared to AO.

Vitamin-D receptor(VDR) genes: T, wild type allele; t, mutant allele; TT, wild type homozygotes: Tt, heterozygote carrier: tt mutant homozygotes: tt - †P<0.02: ††P<0.05 compared to ATB.

Interleukin-1 receptor antagonist (IL-1RA) genes: 11, homozygotes for allele 1; 12, heterozygotes for allele 1 & 2; 22, homozygotes for allele 2. 11 - \$ P <0.01; \$\$ P <0.05 compared to ATB. 11 vs 22 - £ P <0.05 compared to NHS and CT of 11 genotypes. Selected genotypes (high frequency) only studied. Other combinations not determined.

lymphocyte response was observed in active pulmonary tuberculosis patients with 11, 12 and 22 of IL- 1RA genotypes. However, IL-1RA genotype 11 was significantly associated with increased lymphocyte response in normal healthy subjects (P< 0.01) and contacts (P<0.05) when compared to active TB patients with 11 genotype. Moreover, a decreased lymphocyte response was observed in control subjects (NHS & CT) with IL-1RA genotype 22 when compared to 11 genotype of control subjects (P<0.05).

Active tuberculosis patients with the genotype AO (heterozygote carriers) of MBP showed a significantly decreased (P<0.001) tuberculin reactivity when compared to patients with wild type genotype AA (wild type homozygotes) of MBP However, patients with the mutant genotype OO (functional mutant homozygotes) of MBP did not show any marked increase or decrease in the tuberculin reactivity when compared to the genotype AA or AO of MBP (Fig. 2). No difference in the tuberculin reactivity pattern was seen with variant genotypes of vitamin D receptor (VDR) and interleukin-1 receptor antagonist (IL-IRA) genes (Fig.2).

No difference in the tuberculin status was observed between male (19.5 ± 0.5) and female (21.5 ± 0.8) patients. However, female pulmonary TB patients with the vitamin D receptor genotype Tt (heterozygote carrier) showed a higher tuberculin reactivity than male pulmonary TB patients with Tt genotype (P<0.001; Fig. 3). No marked difference in the tuberculin reactive status was seen between male and female pulmonary TB patients positive for either wild type homozygotes (TT) or mutant homozygotes (tt) of VDR.

Discussion

In active pulmonary tuberculosis, the functional mutant homozygotes (FMH) of MBP (00) are associated with a decreased lymphocyte response to *M. tuberculosis* culture filtrate antigen when

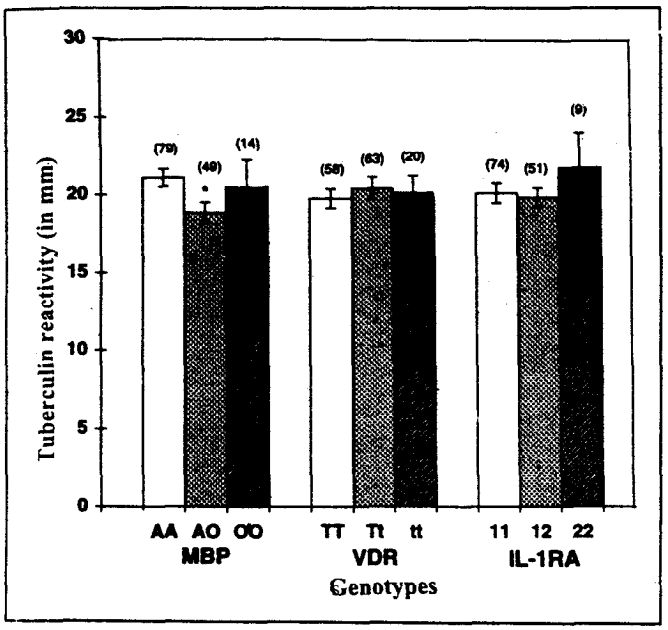

Fig. 2. Influence of variant genotypes of MBP, VDR and IL-1RA genes on tuberculin reactivity in pulmonary TB patients. Results are mean ± SE. *P <0.001 compared to AA. Numbers studied in each group are represented in parentheses.

Mannose binding protein (MBP) genes: A. wild type allele: O, represents the mutant alleles of MBP genes 52, 54 and 57.

Vitamin-D receptor (VDR) genes: T, wild type allele: t, mutant allele.

Interleukin-1 receptor antagonist (IL-IRA) genes: Selected genotypes (high frequency) only studied. Other combinations not determined.

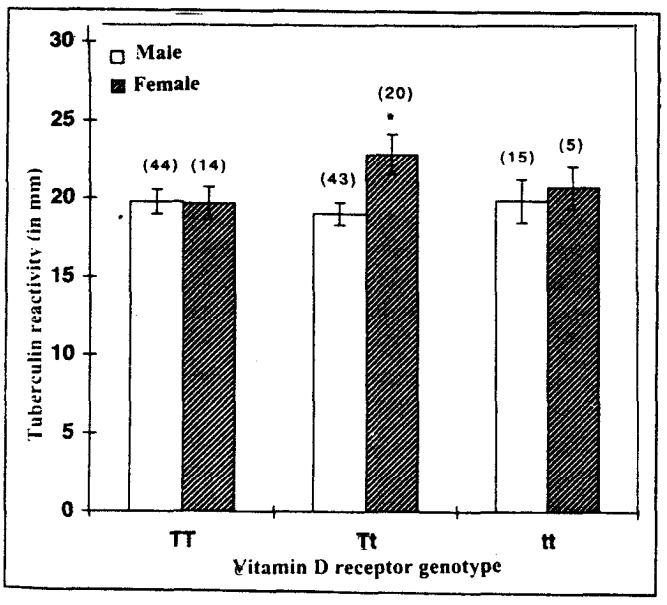

Fig. 3. Influence of vitamin D receptor genotypes on tuberculin reactivity status in male and female pulmonary TB patients. Results arc mean ± SE. Numbers studied in each group are represented in parenthesis. *P <0.001 compared to Tt males.

compared to wild type homozygotes (AA) and heterozygotes (AO) of MBP in active TB patients. Due to lack of mutant homozygotes (OO) among the control subjects (except one normal healthy subject) such a difference could not be compared with the controls. Our recent study[9] in pulmonary TB revealed that functional mutant homozygotes as well as heterozygote carriers of MBP genes are independently associated with the susceptibility to pulmonary TB apart from HLA-DR2. Our earlier studies[17,18] also revealed that HLA-DR2 is associated with susceptibility to pulmonary TB as well as a decreased spontaneous lymphocyte response. In the present study also FMH (OO) of MBP was associated with a decreased antigen induced lymphocyte response. Though FMH is associated with decreased lymphocyte response in pulmonary TB, no such difference was noted in the tuberculin response. The same mutant allele in combination with wild type allele (AO; heterozygote carrier) decreases the tuberculin response to PPD. FMH has been shown to be associated with low serum level of MBP which results in recurrent infections[19,20]. The decreased lymphocyte response found in the present study may be due to impaired macrophage function. MBP has been shown to interact with macrophages through various receptors. In pulmonary TB, FMH of MBP may be associated with low level of serum mannose binding protein which may affect the antigen (glycoprotein components of *M. tuberculosis* culture filtrate antigen) uptake by the macrophages. This in turn may affect antigen presentation by macrophages and antigen recognition by lymphocytes which may lead to decreased lymphocyte response as well as cytokine response. This may affect the CMI response in pulmonary TB. This suggests that the mutant allele either alone or in combination with the wild type allele may be associated with a decreased CMI response which may allow *M. tuberculosis* to establish the infection in a susceptible host and lead to progression of the disease.

The mutant genotype tt of VDR was associated with increased antigen induced lymphocyte response in normal healthy subjects as well as contacts when compared to active TB patients. It has been shown that the mutant VDR genotype tt is associated with increased VDR expression[6]. Moreover, the action of

vitamin D has been shown to be exerted through VDR[6]. Vitamin D activates the macrophages and lymphocytes through vitamin D receptors effectively. This in turn leads to increased production of various proteins and cytokines and other factors which may augment the lymphocyte response to *M. tuberculosis* antigen in control subjects. However, such an increase was not seen in active tuberculosis patients. This may be due to altered cytokine response or other factors which could inhibit or alter the lymphocyte response to *M. tuberculosis* in the active stage of tuberculosis. An increased tuberculin reactivity status was seen. in the vitamin D receptor genotype Tt of female patients with pulmonary TB. Tuberculin reactivity status has been correlated to *M. tuberculosis* infection. Our earlier study[10] suggested that tt genotype is associated with susceptibility to pulmonary tuberculosis in female patients. It has been shown that the allele t plays an Important role in female subjects. The VDR genotype tt is associated with bone mineral density as well as susceptibility to tuberculosis in females[21,22]. Recent studies on VDR genotypes and different types of leprosy[23] and HIV infection (Ali *et al*, personal communication) suggest that the VDR gene polymorphism may be of immunoregulatory importance for many diseases. Further studies on the role of other VDR genes such. as A, a (Apal polymorphism) and B, b (Bsml polymorphism) will explore the immune mechanism of disease susceptibility to tuberculosis.

Though IL-1RA genotype 22 in the control subjects may be associated with decreased lymphocyte response to *M. tuberculosis* antigens when compared to IL-IRA genotype 11, such a decrease was not observed in the patient group with 22 genotype. On the other hand, an increased antigen induced lymphocyte response was seen in patients with 22 genotype when compared to 22 genotype of control subjects as well as patients with 11 genotype. It has been shown that macrophages with heterozygote carriers of IL-IRA allele 2 produce more IL-IRA and less IL-1 α than with other genotypes[8]. Further, 22 genotypes have been shown to be associated with autoimmune diseases[24]. The decreased lymphocyte response associated with IL- 1RA genotype 22 of control subjects may be associated with increased production of IL- 1RA. In turn IL- 1RA may affect the IL- 1 interaction with other cells which may affect the production of other cytokines such as IL-2 which in turn may affect the lymphocyte response to *M. tuberculosis*. The increased or decreased lymphocyte response seen in the patient group may be due to altered level of cytokines produced by the macrophages and lymphocytes.

The present study suggests that the non-MHC genes such as mannose binding protein gene and vitamin D receptor gene along with other MHC and/ or non-MHC genes (multicandidate genes) may regulate or control the CMI response in a susceptible or a resistant host to tuberculosis. Since MBP and VDR genes are associated with the susceptibility to tuberculosis, further studies on the level of cytokines that are associated with the protective immunity may throw more light on the role of host genetics on the immune mechanism of susceptibility/resistance to tuberculosis.

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References

1. Vanham G, Toossi Z, Hirsch CS, Wallis RS, Schwandcr SK. Rich EA. Examining a paradox in the pathogcnesis of human pulmonary tuberculosis : immune activation and suppression anergy. *Tuber Lung Dis* 1991; *78:* 145-58.
2. Turner MW. Mannose-binding lectin : the pluripotcnt molecule of the innate immune system. *Immunol Today* 1996: *17:* 532-40.
3. Ernst JD. Macrophage receptors for *Mycobacterium tuberculosis. Infect Immun* 1998; 66: 1277-81.
4. Rook GAW, Taverne J, Leveton C, Steele J. The role of gamma-interferon, vitamin D, metabolites and tumour necrosis factor in the pathogenesis of tuberculosis. *Immunology* 1987: *62:* 229-34.
5. Rook GAW. The role of vitamin D in tuberculosis. *Am Rev Respir Dis* 1988; *138:* 768-70.
6. Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV. Prediction of bone density from vitamin D receptor alleles. *Nature,* 1994; *367:* 284-7.
7. Arend WP, Malyak M, Guthridge CJ, Gabay C. Interleukin-1 receptor antagonist : role in biology. *Annu Rev Immunol* 1998; *16:* 27-55.

8. Danis V.A, Millington M, Hyland VJ, Grennan D. Cytokine production by normal human monocytes : inter-subject variation and relationship to an IL- 1 receptor antagonist (IL- 1Ra) gene polymorphism. *Clin Exp Immmunl* 1995; *99:* 303-10.

9. Selvaraj P, Narayanan PR, Reetha AM. Association of functional mutant homozygotes of the mannose binding protein gene with susceptibility to pulmonary tuberculosis in India. *Tuber Lung Dis* 1999; *79:* 221-7.

10. Selvaraj P, Narayanan PR, Reetha AM. Association of vitamin D receptor genotypes with the susceptibility to pulmonary tuberculosis in female patients and resistance in female contacts. *Indian J Med Res* 2000; *111:* 172-9.

11. de Vries RRP. An immunogenetic view of delayed type hypersensitivity. *Tubercle* 1991; *72:* 161-7.

12. Qttenhoff THM, Torres P, de las Aguas JT, Fernadez R, van Eden W, de Vries RRP, et al. Evidence for an HLA-DRC associated immune-response gene for *Mycobacterium tuberculosis.* A clue to the pathogenesis of rheumatoid arthritis? *Lancet* 1986: *ii:* 310-2.

13. Bothamley GH, Gibbs JH, Beck JS, Schreuder GMT, de Vrie RRP, Grange JM. *et al.* Delayed hypersensitivity and HLA in smear-positive pulmonary tuberculosis. *Int Arch Allergy Immunol* 1995; *106:* 38-45.

14. Selvaraj P, Reetha AM, Uma H, Xavier T, Janardhanam B, Prabhakar R. *et al.* Influence of HLA-DR and -DQ phenotypes on tuberculin reactive status in pulmonary tuberculosis patients, *Tuber Lung Dis* 1996; *77:* 369-73.

IS Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol regent. *J Biol Chem* 1951; *193:* 265-75.

16. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells *Nucleic Acids Res* 1988; *16:* 1215.

17. Selvaraj P, Uma H, Reetha AM, Kurian SM, Xavier T, Prabhakar R. et al. HLA antigen profile in pulmonary tuberculosis patients and their spouses. *Indian J Med Res* 1998: *107:* 155-8.

18. Selvaraj P, Uma H, Reetha AM, Xavier T, Prabhakar R, Narayanan PR. Influence of HLA-DR2 phenotype on humoral immunity and lymphocyte response to *Mycobacterium tuberculosis* culture filtrate antigens in pulmonary tuberculosis. *Indian J Med Res* 1998; 107: 208-17.

19. Soothill JF, Harvey BA. Defective opsonization. A common immunity deficiency. *Arch Dis Child* 1976; *51:* 91-9.

20. Richardson VF, Larcher VF, Price JF. A common congenital immunodeficiency predisposing to infection and atopy in infancy *Arch Dis Child* 1983; *58:* 799-802.

21. Eisman JA. Vitamin D receptor gene variants : implications for therapy. *Curr Opin Genet Dev* 1996: *6:* 361-5

22. Sainz J, Van Tornout JM, Loro ML, Sayre J, Roe TF, Gilsanz V. Vitamin D receptor gene polymorphisms and bone density in prepubertal American girls of Mexican descent. *N Engl J Med* 1997; *337:* 77-82.

23. Roy S, Frodsham A, Saha B, Hazra SK, Mascie-Taylor CGN, Hill AVS. Association of vitamin D receptor genotype with leprosy type. *J Infect Dis* 1999; *179:* 187-91.

24. Mansfield JC, Holden H, Tarlow JK, Giovine FSD, McDowell TL, Wilson AC. *et al.* Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. *Gastroenterology* 1994: *106:* 637-42.

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