Polymorphisms of the *NRAMP1* gene: Distribution and susceptibility to the development of pulmonary tuberculosis in the Greek population

Authors' Contribution:
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B Data Collection
C Statistical Analysis
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Summary

Background: Ample evidence suggests that host genetic factors affect human susceptibility to tuberculosis. The natural resistance–associated macrophage protein 1 (*NRAMP1*) gene seems to play a role in the pathophysiology of a number of intracellular infections, including mycobacteria. A case-control study was conducted in the Greek population to determine whether *NRAMP1* polymorphisms affect the susceptibility to development of overt pulmonary tuberculosis.

Material/Methods: *NRAMP1* polymorphisms (3'UTR, D543N, INT4) were evaluated among 142 patients with culture-positive pulmonary tuberculosis and 144 ethnically matched healthy controls having latent *M. tuberculosis* infection. Patients with human immunodeficiency virus infection were excluded.

Results: Out of the 3 *NRAMP1* polymorphisms, a trend of increased incidence of INT4 polymorphism was found in the patients' group compared to the control group. A lack of association was observed between the 2 groups as far as the other 2 polymorphisms (D543N, 3'UTR) are concerned. INT4-CC homozygotes were found to have a higher risk to develop pulmonary tuberculosis compared to GG homozygotes (p=0.022). An increased incidence G/TGTG/C genotype combination was found in the patients' group as compared to controls. G/TGTG/C genotype combination was associated with a 36% higher risk of developing pulmonary tuberculosis (p=0.004) compared to the baseline expression of G/TGTG/G combination.

Conclusions: INT4-*NRAMP1* polymorphism may have a role in the development of culture-positive pulmonary tuberculosis after an initial *M. tuberculosis* latent infection. The possible role of INT4-*NRAMP1* polymorphism in the development of active pulmonary tuberculosis needs further investigation.

key words: ***NRAMP1/SLC11A1* gene** • tuberculosis • polymorphisms • ethnic groups

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BACKGROUND

Tuberculosis (TB) remains the most widespread and deadly infectious disease worldwide, and it has been recently declared as a great emergency by the World Health Organization (WHO) [1]. Unfortunately, 8.9–9.9 million new cases of TB were reported in 2008, while 1.1–1.7 million human immunodeficiency virus (HIV)-negative patients died from this disease [2]. The factors which are mainly responsible for this evolving incidence are: (i) the migration of individuals from regions with high TB incidence to those with low incidence, (ii) the increasing number of HIV-positive individuals, and (iii) the emergence of multidrug-resistant TB due to unwise use of antibiotics [1,2]. In a total population of approximately 11 million people, the incidence of TB in Greece is estimated at 18/100,000 persons and the mortality rate at 2 /100,000 annually [2–4].

The spread of *Mycobacterium tuberculosis* in the majority of humans is inhibited after the development of an effective immune response. Less than 10% of infected persons will eventually develop clinical disease [5]. Interestingly, only a minority of these patients have an identifiable risk factor, such as HIV infection, advanced age, alcohol abuse, chronic corticosteroid therapy, diabetes and poor nutrition/socio-economic status [6,7]. In the remaining patients, a complex interaction of genetic and environmental factors is possibly the cause. Studies in twins strongly suggest that host genetic factors play a major role in the susceptibility to development of TB [8]. Intense efforts are therefore being made to discover any possible genetic factors that may have a role in the host defense system and explain the increased susceptibility to overt *M. tuberculosis* infection. In 1981 a gene was found in mice that played an essential role in the determination of resistance to mycobacteria and other intracellular pathogens [9]. This gene was named natural resistance-associated macrophage protein 1 (*Nramp1*) [9]. The human homologue of the *Nramp1* gene, designated *NRAMP1*, now known as *SLC11A1*, is located on the long arm of chromosome 2 (2q35). *NRAMP1* is considered as a strong candidate gene for human resistance to TB. NRAMP1 protein seems to play an important role in regulation of cytoplasmic cation levels, especially iron. Iron is an essential mycobacterial nutrient, and has a major role in generating reactive oxygen and nitrogen intermediates in macrophages; hence, it affects the intracellular growth of mycobacteria [10,11]. Although it was believed that the gene is expressed solely in reticuloendothelial cells, it was recently shown to be expressed in CD11c⁺ dendritic cells, and exerts its pleiotropic effects on cytokine transcription, major histocompatibility complex (MHC) class II molecule expression, and presentation of protein antigens to T lymphocytes [12].

The possible association of *NRAMP1* polymorphisms with susceptibility to development of TB has been studied in different countries. The results of these studies among different ethnic groups have been controversial. A case-control study, conducted in West Africans, showed a positive association of the gene with pulmonary TB [13]. A similar study, conducted in Koreans, detected a significant association for the 3'UTR polymorphism, but not for the INT4 locus [14]. On the other hand, studies conducted in Denmark, Mexico, Morocco, Japan, Brazil and China suggested that the gene is not associated with an increased risk for developing TB

[15–17]. In a study conducted in the Chinese population, *NRAMP1* allelic polymorphisms at the INT4 and D543N *loci* were each significantly associated with severe forms of pulmonary TB [17]. The aim of the present study was to explore the association between *NRAMP1* polymorphisms and the susceptibility to develop active pulmonary TB after a latent *M. tuberculosis* infection, in the Greek population.

MATERIAL AND METHODS

Study population

Patients' group

Inclusion criteria

The inclusion criteria for the patients' group were: (i) Greek origin; (ii) all patients should be genetically unrelated; and (iii) diagnosis of pulmonary TB based on suggestive radiological findings, a microscopic examination of sputum smears from an experienced microscopist, and documented in all patients with the presence of positive sputum cultures for *M. tuberculosis* infection. Written informed consent to participate was obtained from all patients who were included.

Exclusion criteria

The exclusion criteria for the patients' group were: (i) presence of extrapulmonary TB; (ii) patients known to have an HIV infection were not recruited (all the other patients who agreed to participate were screened for HIV antibodies and HIV-positive patients were not enrolled in the study); and (iii) presence of any immunodeficiency syndrome or systemic disease.

A 5 ml blood sample was drawn from 142 Greek patients with pulmonary TB who were hospitalized in the Sotiria General Hospital of Thoracic Diseases from January 2004 until December 2008. The ethics committee of Sotiria General Hospital, Athens, Greece, accepted the study protocol. Currently, in the Athens area of Greece, Sotiria General Hospital of Thoracic Diseases is the health institution where the majority of TB cases are diagnosed and monitored. The sample size was considered to be adequate for the study, taking into consideration the size of the Greek population and the incidence of TB in Greece [2]. Moreover, during the study period, cases of pulmonary TB were diagnosed, but were excluded from the study because most of these TB cases were diagnosed in immigrants of foreign origin and due to the patient's co-morbidities.

Control group

Inclusion criteria

The inclusion criteria for the control group were: (i) ethnically matched volunteers who visited Sotiria Hospital for a routine check-up; and (ii) the presence of a latent *M. tuberculosis* infection. Latent *M. tuberculosis* infection was defined as a positive tuberculin skin and at least 2 negative sputum cultures, combined with the absence of suggestive radiological findings for pulmonary TB. Written informed consent to participate was obtained from all controls who were included.

Briefly, healthy subjects who visited Sotiria Hospital for a routine check-up were enrolled. A 5 ml blood sample was drawn from every healthy volunteer, and was handled identically to the patients' group. In all controls a tuberculin skin test was performed. Specifically, 0.1 ml of purified protein derivative (PPD), which contained 5 tuberculin units, was injected intradermally in the volar surface of the forearm. A 27-gauge needle on a tuberculin syringe was used. The transverse width of induration in millimetres (mm) at the skin test site was measured after 48-72 hours. A positive tuberculin skin test was defined as skin transverse width ⩾ 12 mm. In all of the controls enrolled, a chest-X-ray (CXR) and sputum cultures for *M. tuberculosis* were obtained in order to rule out active disease. One hundred forty-four ethnically matched volunteers with latent *M. tuberculosis* infection were included.

Exclusion criteria

The exclusion criteria for the control group were: (i) HIV-positive subjects; (ii) history of prior TB; (iii) evidence of current or prior TB in the CXR; and (iv) presence of any immunodeficiency syndrome or systemic disease.

NRAMP1 genotyping

DNA samples were extracted from human whole blood collected into tubes containing EDTA. The kit used for the extraction was PureLink™ Genomic DNA kit Catalog no.s K1820-01, K1820-02, K1821-04, from Invitrogen. The *NRAMP1* polymorphisms typed were: (i) a single-nucleotide change in intron 4 (INT4) (469+14G/C); (ii) D543N, a non-conservative single-base substitution at codon 543, which changes aspartic acid to asparagine; and (iii) 3'UTR, a TGTG deletion in the 3'untralsated region (1729+55del4). The PCR primers for the INT4 polymorphism were 5'CTCTGGCTG AAGGCTCTCC3' and 5'TGTGCTAT CAGTTGAGCCTC3'. The primers for D543N and 3'UTR were 5'GCATCT CCCCAATTCATGGT3' and 5'AAC TGTCCCACTCTATCCTG3', respectively.

Real-time PCR studies were performed in a LightCycler® 480 Instrument, Roche. For each of the 3 polymorphisms analyzed, a LightSNip kit was designed from the constructor company, TIB-MolBiol, Germany. Every reagent vial contained all primers and probes to run 96 Lightcycler reactions. The PCR reaction mixture contained 14.4–10.4 µl PCR grade H20, 1.0 µl Reagent Mix 2.0 µl Lightcycler FastStart DNA Master Hyprobe 1.6 µl MgCl$_2$ and 1.0–5.0 µl DNA template. The LightCycler® 480 Instrument was programmed according to the manufacturer's parameters.

Statistical analysis

Initially, variants at 3 polymorphisms (3' UTR, INT4, D543N) in the *NRAMP1* gene were compared between tuberculosis cases and controls with Fisher's Exact test. To assess the differences in demographic characteristics, we used chi-square (for categorical data) and t test (for continuous data). Deviations from Hardy-Weinberg Equilibrium (HWE) were assessed using the chi-square test. Subsequently, the 3 *NRAMP1* polymorphisms were each independently associated with tuberculosis through logistic regression analysis after adjustment for age and sex. The SAS statistical package

Table 1. Demographic characteristics and incidence of *NRAMP1* gene polymorphisms (3'UTR, D543N, INT4), between the two groups.

Variables	pTB patients (n=142)		Controls (n=144)		P-value*
Gender					NS
Male (%)	98	(69.0)	94	(65.3)	
Female (%)	44	(31.0)	50	(34.7)	
Age (years) ±SD	54.32±16.44		59.63±10.77		0.001
D543N					0.743
G/G (%)	138	(97.2)	139	(96.5)	
G/A (%)	4	(2.8)	5	(3.5)	
3'UTR					1.00
TGTG/TGTG (%)	137	(96.5)	139	(96.5)	
TGTG/del (%)	4	(2.8)	4	(2.8)	
Del/del (%)	1	(0.7)	1	(0.7)	
INT4					0.058
G/G (%)	84	(59.2)	93	(64.6)	
G/C (%)	48	(33.8)	49	(34.0)	
C/C (%)	10	(7.0)	2	(1.4)	

* P-values derived from: Chi-square for gender, t-test for age and Fisher's exact test for gene polymorphisms; NS – Not statistically significant; pTB – Pulmonary tuberculosis.

(Version 9.1, SAS Institute Inc, Cary, NC) was used to analyze the data. Significance level was set at p=0.05.

RESULTS

All patients who were enrolled had a culture-positive pulmonary TB, combined with suggestive radiological findings. Positive acid-fast bacilli (AFB) sputum smear for *M. tuberculosis* was observed in 108 (76%) patients; the remaining 34 (24%) patients had a negative AFB smear test. The demographic characteristics and the frequency of the 3 *NRAMP1* polymorphisms (3'UTR, D543N, INT4) between patients and controls are shown in Table 1. There was no evidence for deviation from HWE regarding the polymorphisms D543N (p=0.07) and INT4 (p=0.08), whereas the 3'UTR (p=29.11) was found to deviate significantly from HWE.

The *NRAMP1* allelic frequencies for the D543N and 3'UTR variants were not found to differ significantly between patients with pulmonary TB and controls. D543N associated G/G and G/A variants were observed in 138 and 4 samples in the patients' group, respectively. In controls, D543N-associated G/G and G/A genotypes were observed in 139 and 5 samples, respectively. The genotype A/A was absent in both groups. In the patients' group, the 3'UTR-associated TGTG/TGTG, TGTG/del and del/del variants were observed in 137, 4 and 1 sample, respectively. In the control group, the numbers of samples with positive 3'UTR

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Table 2. Logistic regression-derived odds ratios (ORs) and 95% confidence intervals (95% CIs) for the risk of developing pulmonary tuberculosis according to the variant of each of the three allele polymorphisms, after adjustment for gender and age.

Variable	ORs	95% CI	P-value
D543N			
G/G	Baseline		
G/A	1.02	0.22–4.70	0.977
3'UTR			
TGTG/TGTG	Baseline		
TGTG/del	1.26	0.25–6.37	0.780
Del/del	0.57	0.02–18.50	0.754
INT4			
G/G	Baseline		

Table 3. Frequency of *NRAMP1* genotype combinations among 1136 alleles derived from the 142 patients with pulmonary tuberculosis and 1152 alleles derived from 144 controls.

Variables	pTB patients		Controls		P-value
G/TGTG/G	842	(74.1)	911	(79.0)	0.005*
G/TGTG/C	262	(23.1)	209	(18.1)	0.004*
G/del/G	10	(0.9)	11	(1.0)	0.852*
A/del/G	6	(0.5)	11	(1.0)	0.235*
A/TGTG/G	6	(0.5)	7	(0.6)	0.800*
G/del/C	6	(0.5)	1	(0.1)	0.068**
A/TGTG/C	2	(0.2)	1	(0.1)	0.622**
A/del/C	2	(0.2)	1	(0.1)	0.622**

* P-value derived from chi-square test; ** P-value derived from Fisher's Exact test; pTB – Pulmonary tuberculosis.

Table 4. Logistic regression-derived odds ratios (ORs) and 95% confidence intervals (95% CIs), estimating the risk of developing pulmonary tuberculosis, among the five most common *NRAMP1* genotype combinations.

Variable	ORs	95% CI	P-value
G/TGTG/G	Baseline	–	–
G/TGTG/C	1.36	1.11–1.66	0.004
G/del/G	0.98	0.42–2.33	0.970
A/del/G	0.59	0.22–1.60	0.301
A/TGTG/G	0.93	0.31–2.77	0.893

variants were 139, 4 and 1 sample, respectively. However, we identified an increased incidence of INT4 polymorphism in the patients' group compared to the control group, which was close to the borderline of statistical significance (p=0.058).

Three separate logistic regression analyses were performed, after adjustment for age and sex, in order to further investigate the possible association of TB patients with the allelic frequencies of the 3 *NRAMP1* polymorphisms (Table 2). No significant association was found in patients with pulmonary TB for the D543N and 3'UTR allele variants. A significant association was found between the allele variants for the INT4-*NRAMP1* polymorphism. Specifically, when GG allele genotype was used as baseline value, CC homozygotes were found to be at higher risk for developing pulmonary TB (OR=7.31, 95% CI: 1.33-40.17, p=0.022). GC heterozygotes were not found to differ significantly from GG homozygotes (p=0.628).

A more detailed analysis was performed by creating 8 categories of genotype combinations: G/TGTG/G, G/TGTG/C, G/del/G, A/del/G, A/TGTG/G, G/del/C, A/TGTG/C and A/del/C (Table 3). G/TGTG/G was the main genotype combination observed. Significant differences among the frequencies between patients and controls were found regarding G/TGTG/G and G/TGTG/C genotype combinations. Specifically, lower expression of G/TGTG/G combination (p=0.005) was found in the patients' group compared to the control group, while higher expression of the G/TGTG/C combination (p=0.004) was found in the patients' group compared to the controls.

From a total of 8 genotype combinations, 5 (G/TGTG/G, G/TGTG/C, G/del/G, A/del/G, A/TGTG/G) were included in a multivariate model (Table 4). The remaining 3 combinations (G/del/C, A/TGTG/C, A/del/C) were excluded because of their extremely low frequencies. G/TGTG/C was found to be associated with a 36% higher risk for developing pulmonary TB (OR=1.36; 95% CI: 1.11–1.66; p=0.004)

compared to the baseline expression of G/TGTG/G. An association between pulmonary TB and G/del/G, A/del/G and A/TGTG/G genotype combinations, compared to the baseline expression of G/TGTG/G, was not observed.

DISCUSSION

To our knowledge this is the first study in Greece that examines the possible association of *NRAMP1* polymorphisms and the susceptibility to develop culture-positive pulmonary TB. Our study demonstrated that there was not any significant difference in the incidence of *NRAMP1* D543N and 3'UTR polymorphisms between patients with pulmonary TB and ethnically matched healthy controls having latent *M. tuberculosis* infection. An increased incidence of INT4-*NRAMP1* polymorphism was found in the patients' group, which was close to the borderline of statistical significance.

A positive association of 3'UTR variant allele and the susceptibility to develop TB was reported in a small Caucasian population [18]. However, several studies performed in European populations suggested no statistically significant association between *NRAMP1* polymorphisms and the susceptibility to develop TB. This was also confirmed in the biggest meta-analysis of published trials to date [19]. In

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this analysis, the odds ratios (ORs) among 3'UTR, D543N, INT4 locus and TB susceptibility, in the European population, were 1.81 (95%CI: 0.66–4.93; p=0.25), 1.88 (95%CI: 0.75–4.67; p=0.18) and 0.87 (95%CI: 0.61–1.22; p=0.41), respectively [19]. It is believed that the European origin population has a high level of "genetical" resistance to *M. tuberculosis* infection, due to natural selection over the last 300 years [20,21]. The lower proportion of TB cases in the European population, compared to Asian and African populations, could be another reason for the lack of association [19]. We found a lack of correlation among D543N and 3'UTR *NRAMP1* polymorphisms and an increased susceptibility to develop pulmonary TB, according to results of the majority of studies in European populations.

Although our results are close to statistical significance, there is a trend for a higher incidence of INT4-*NRAMP1* polymorphism in patients with pulmonary TB, compared to ethnically matched volunteers with latent *M. tuberculosis* infection. This possible association appears for the first time in a European population, and can be possibly attributed to the diverse genetic background among various ethnic populations of the same descent. Ethnic or racial backgrounds can certainly be responsible for some variations observed [18]. Specifically, the INT4-*NRAMP1* polymorphism can be found in approximately 20% of the Chinese population, whereas the frequency in white Europeans is about 50%, with significant variation among different European populations [17,19].

The INT4-CC homozygotes variants were found to have an increased risk for developing pulmonary TB, compared to their corresponding common GG alleles. When a more detailed analysis was performed by creating 8 categories of genotype combinations, higher expression of G/TGTG/C combination (p=0.004) was found in the patient's group as compared to the controls. This combination was associated with a 36% higher risk for developing pulmonary TB (OR=1.36; 95% CI: 1.11–1.66; p=0.004) compared to the baseline expression of G/TGTG/G. However, the limited number of patients and controls found to carry the INT4-CC genotype must be taken into consideration in the interpretation of our results. Whether the incidence of INT4-CC variant or any *NRAMP1* genotype combination is correlated to the development of active disease needs further investigation.

Most of the studies exploring the possible role of *NRAMP1* polymorpisms in the development of TB did not distinguish between susceptibility to infection with *M. tuberculosis* and susceptibility to progression to active disease, since the control groups consisted of healthy volunteers without active disease. Our study design distinguishes between susceptibility to infection with *M. tuberculosis* and susceptibility to progression in an active *M. tuberculosis* pulmonary disease. All volunteers in the control group had a latent *M. tuberculosis* infection without developing the disease. This fact suggests that INT4-*NRAMP1* polymorphisms may have a role in the development of overt pulmonary disease after a latent *M. tuberculosis* infection. The main limitations of our study are the lack of data in both study groups that can be associated with a higher risk of developing pulmonary TB (alcohol abuse, chronic corticosteroid therapy, diabetes, poor nutrition/socio-economic status), and the relatively small sample size of the population enrolled.

CONCLUSIONS

INT4-*NRAMP1* polymorphism may be associated with a higher rate of culture-positive pulmonary TB in the Greek population compared to ethnically matched healthy controls with latent *M. tuberculosis* infection. A lack of association was observed for the other 2 *NRAMP1* polymorphisms (D543N, 3'UTR). INT4-*NRAMP1* CC homozygotes were found to have a higher risk compared to GG homozygotes. An increased incidence of G/TGTG/C genotype combination was found in the patients' group compared to controls. G/TGTG/C genotype combination was associated with a 36% higher risk for developing pulmonary TB compared to the baseline expression of G/TGTG/G genotype combination. The possible role of INT4-*NRAMP1* polymorphism in the development of active pulmonary TB, after an initial latent *M. tuberculosis* infection, needs further investigation.

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Conflict of interest

All authors declare no conflict of interest in relation to this study.

REFERENCES:

1. Al-Tawfiq JA: Multifocal systemic tuberculosis: the many faces of an old nemesis. Med Sci Monit, 2007; 13(4): CS56–60

2. WHO Report 2009 Global Tuberculosis Control. A short update to the 2009 report

3. Papaetis GS, Pefanis A, Solomon S et al: Asymptomatic stage-I sarcoidosis complicated by pulmonary tuberculosis: a case report. J Med Case Reports, 2008; 2: 226

4. Samara I, Bakakos P, Orphanidou D et al: Sputum adenosine deaminase activity in patients with pulmonary tuberculosis and lung cancer. Adv Clin Exp Med, 2007; 16: 533–35

5. Murray CJ, Styblo K, Rouillon A: Tuberculosis in developing countries: burden, intervention and cost. Bull IUATLD, 1990; 65: 6–24

6. Güneylioglu D, Yilmaz A, Bilgin S et al: Factors affecting delays in diagnosis and treatment of pulmonary tuberculosis in a tertiary care hospital in Istanbul, Turkey. Med Sci Monit, 2004; 10(2): CR62–67

7. Skyba P, Kluchova Z, Joppa P et al: Nutritional status in relation to respiratory impairment and systemic inflammation in patients with acute exacerbations of COPD. Med Sci Monit, 2009; 15(10): CR528–33

8. Comstock GW: Tuberculosis in twins: a re-analysis of the Prophit survey. Am Rev Respir Dis, 1978; 117: 621–24

9. Gros P, Skamene E, Forget A: Genetic control of natural resistance to Mycobacterium bovis (BCG) in mice. J Immunol, 1981; 127: 2417–21

10. Cellier M, Govoni G, Vidal S et al: Human natural resistance-associated macrophage protein: cDNA cloning, chromosomal mapping, genomic organization, and tissue-specific expression. J Exp Med, 1994; 180: 1741–52

11. Søborg C, Andersen AB, Madsen HO et al: Natural resistance-associated macrophage protein 1 polymorphisms are associated with microscopy-positive tuberculosis.. J Infect Dis, 2002; 186: 517–21

12. Stober CB, Brode S, White JK et al: Slc11a1, formerly Nramp1, is expressed in dendritic cells and influences major histocompatibilty complex class II expression and antigen-presenting cell function. Infect Immun, 2007; 70: 5059–67

13. Bellamy R, Ruwende C, Corrah T et al: Variations in the NRAMP1 gene and susceptibility to tuberculosis in West Africans. N Engl J Med, 1998; 338: 640–44

14. Ryu S, Park YK, Bai GH et al: 3'UTR polymorphisms in the NRAMP1 gene are associated with susceptibility to tuberculosis in Koreans. Int J Tuberc Lung Dis, 2000; 4: 577–80

15. Niño-Moreno P, Portales-Pérez D, Hernández-Castro B et al: P2X7 and NRAMP1/SLC11 A1 gene polymorphisms in Mexican mestizo patients with pulmonary tuberculosis. Clin Exp Immunol, 2007; 148: 469–77

16. El Baghdadi J, Resmus N, Benslimane A et al: Variants of the human NRAMP1 gene and susceptibility to tuberculosis in Morocco. Int J Tuberg Lung Dis, 2003; 7: 599–602

17. Zhang W, Shao L, Weng X et al: Variants of the natural resistance-associated macrophage protein 1 gene (NRAMP1) are associated with severe forms of pulmonary tuberculosis. Clin Infect Dis, 2005; 40: 1232–36

18. Liu J, Fujiwara TM, Buu NT et al: Identification of polymorphisms and sequence variants in the human homologue of the mouse natural resistance-associated macrophage protein gene. Am J Hum Genet, 1995; 56: 845–53

19. Li HT, Zhang TT, Zhou YQ et al: SLC11A1 (formerly NRAMP1) gene polymorphisms and tuberculosis susceptibility: a meta-analysis. Int J Tuberc Lung Dis, 2006; 10: 3–12

20. Stead WW: Genetics and resistance to tuberculosis. Could resistance be enhanced by genetic engineering? Ann Intern Med, 1992; 116: 937–41

21. Ezzeldin N, Saad NE, Ezzeldin H, Mohsen M: Variation in serum level of IL-2 and IL-8 in lung fibrosis. Arch Med Sci, 2008; 4(1): 26–31