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Analysis of *IL-4* promoter and VNTR polymorphisms in Thai patients with pulmonary tuberculosis

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Abstract. Tuberculosis (TB) is a leading cause of morbidity and mortality in Thailand. Cytokines play important roles in defense against *Mycobacterium tuberculosis* infection. Interleukin (IL)-4 is one of the anti-inflammatory cytokines and has been found to be elevated in TB patients. The common polymorphisms in *IL-4* gene, including *IL-4*-590C/T, *IL-4*-33C/T, and *IL-4*-variable number of tandem repeats (VNTR) intron 3 have been reported to be associated with risk for some diseases. The purpose of this study was to investigate possible associations between the above mentioned three common functional polymorphisms in the *IL-4* gene in patients with pulmonary tuberculosis (PTB) in a Thai population. Forty three patients with PTB and 90 healthy control subjects were studied. The three common polymorphisms of the *IL-4* gene were determined using polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism (PCR-RFLP). The allele and genotype frequencies of *IL-4* -590 C/T, -33 C/T, VNTR intron 3 polymorphisms did not show significant differences between PTB patients and healthy controls (genotype: p=0.88, p=0.92, p=0.40; allele: p=0.38, p=0.44, p=0.53, respectively). However, the allele distribution of the *IL-4* -590 C, -33 C, and VNTR R3 was higher among PTB patients (25.58%, 25.58%, 25.58%, respectively) than among control subjects (20%, 20.48%, 19.44%, respectively). This may suggest that *IL-4*-590C/T, -33C/T and VNTR intron 3 might play a role in susceptibility to PTB. A larger cohort may possibly help conclude our findings.

INTRODUCTION

Tuberculosis (TB) remains a major public health problem. According to a World Health Organization (WHO) report, the disease caused by *Mycobacterium tuberculosis* was responsible for 6.3 million new cases worldwide and 1.3 million deaths annually (WHO, 2017). Thailand is one of the 22 countries in the world with highest TB burden. Thailand has a population of nearly 67 million, among whom there are approximately 93,000 new TB cases each year and an estimated TB prevalence of nearly 130,000 cases (WHO, 2018). It has been reported that approximately 10% of the population infected by *M. tuberculosis* will develop clinical TB (Arend *et al.*, 2001). The differences in host immunity may affect those who does and does not develop clinical TB. Thus, differences in the genetic background may play a role in the susceptibility to TB infection (Hill, 2001; Moller & Hoal, 2010; Vannberg *et al.*, 2011).

Immune responses to TB are regulated by interactions between antigen presenting cells and T helper (Th) lymphocytes. After Th cells are activated, they secrete pro-

inflammatory cytokines (T helper type 1 response: Th1) in the early phase and subsequently secrete anti-inflammatory cytokines (T helper type 2 response: Th2). Previous studies demonstrated that dysregulation in the balance between Th1 (typified by the secretion of cytokines interleukin (IL)-1β, IL-6, IL-12, interferon (IFN)-γ and tumor necrosis factor (TNF)- α) and Th2 (typified by IL-4, IL-10 cytokines) was associated with enhanced immune-pathogenesis and tuberculosis severity (Dlugovitzky *et al.*, 1997; Hasan *et al.*, 2009).

IL-4, a multifunctional pleiotropic cytokine, plays important roles as a mediator and modulator of immune and inflammatory responses (Durie *et al.*, 1994). Any alteration in the secretion and function of IL-4 can lead to weakened immune responses, and thus increase the susceptibility to infections such as TB (Jiang *et al.*, 2000; Kahnert *et al.*, 2006). Moreover, IL-4 is encoded by the *IL-4* gene which is highly polymorphic, where genetic polymorphisms in this gene might be the major contributor in tuberculosis susceptibility, or conversely, resistance.

The human *IL-4* gene is located on chromosome 5q31-q33 and consists of 25 kilo base pairs (Marsh *et al.*, 1994). The three common functional *IL-4* polymorphisms include-590C/T (rs2243250) and -33C/T (re2070874) in the promoter region, as well as a 70 base pairs (bp) variable number of tandem repeats (VNTR) (rs79071878) in intron 3 have been shown to play a potential role in the outcome of several diseases (Jha *et al.*, 2012; Jeronimo *et al.*, 2007; Birbian *et al.*, 2014). The -590T, -33T, and VNTR R2 polymorphisms lead to more responsiveness to transcription resulting in IL-4 over-production (Rosenwasser *et al.*, 1995; Luoni *et al.*, 1995; Nakashima *et al.*, 2002). This over-production of IL-4 thereby alters the Th1 and Th2 balance by upregulation of the Th2-type response and downregulation of the Th1-type response.

The impact of *IL-4* gene polymorphisms on the susceptibility to TB infection has been investigated, but the results reported have been inconsistent. In a study in Iranians, *IL-4* -590C/T C allele and the C/T genotypes were found to be significantly increased in pulmonary tuberculosis (PTB) patients as compared to that seen in controls, while the -1098G/T and -33C/T were not found to be associated with susceptibility to TB (Amirzargar *et al.*, 2006). A study in Russians showed a relationship between susceptibility to tuberculosis and the presence of the *IL-4*-590 C allele and CC and CT genotypes (Naslednikova *et al.*, 2009). Similarly, a study in Indians demonstrated that the presence of the *IL-4*-590 CC genotype was significantly higher in TB patients than in controls (Sivangala *et al.*, 2014). Another study in south Indians revealed a significantly increased frequency of *IL-4* -590 CT genotype in PTB patients and the CC genotype in control group (Vidyarani *et al.*, 2006). These studies suggested that, *IL-4*-590C/T gene polymorphisms may be associated with tuberculosis. Conversely, a study in Chinese, Brazilian and Turkish subjects did not find any association between *IL-4* -590 C/T and TB (Qi *et al.*, 2014; Milano *et al.*, 2016; Ulger *et al.*, 2013). Similarly, another study in south Indians revealed no difference in the genotype frequencies of *IL-4* VNTR intron 3 between normal healthy subjects and PTB patients (Selvaraj *et al.*, 2008).

Presently, there were no case-control base association studies conducted to investigate the relationship between common functional *IL-4* gene polymorphisms and PTB susceptibility in Thai people living in central area of Thailand. Therefore, we aimed to investigate the possible association between three regulatory *IL-4* polymorphisms namely –590C/T (rs2243250) and –33C/T (rs2070874) in the promoter region, and 70 bp VNTR in intron 3 (rs79071878) and PTB susceptibility in Thai people living in the central area of Thailand.

MATERIALS AND METHODS

Study Groups

All patients were recruited from HRH princess Maha Chakri Sirindhorn Medical Center, Faculty of Medicine, Srinakharinwirot

University. Srinakharinwirot University has 2 campuses. The first campus is located at Watana district, Bangkok city. The second campus is located at Ongkharuck district, Nakornnayok province. Ongkharuck district is about 75 kilometers far from Bangkok and located at central part of Thailand. Stored anonymous DNA samples from pulmonary tuberculosis (PTB) cases from a previous study (Kulpraneet *et al.*, 2015), which included 43 patients with newly diagnosed PTB were used to elucidate in this study. All of those patients were either smear/culture positive or with clinical-radiological and histological evidences for PTB. Their mean age was 48.22 (range 18-84) years for men and 49.80 (range 25-84) for women, and male/female ratio 28/15. Only patients, who had no sign of immunodeficiency, autoimmune diseases, allergic diseases and diabetes mellitus in their history records, were included in this study. The healthy control subjects (HCS) were students, and laboratory personnel who were clinically normal and were willing to participate in the study. Among the HCS, 30 were males (mean age 34.73, range 21-59) and 60 females (mean age 34.32, range 19-75). This study was approved by the Faculty of Medicine, Srinakharinwirot University, Ethical Review Committee (SWUEC/E-294/2559). It was conducted according to the principles established in the Declaration of Helsinki. An informed consent was obtained from all the participants.

DNA purification

Anonymous genomic DNA samples from patients with PTB stored at minus 20°C and isolated as previously described was studied (Kulpraneet *et al.*, 2015). Genomic DNA from HSC saliva was isolated using a commercial DNA purification kit (Roche-applied-science, Germany), according to the manufacturer's instructions with some modification. Briefly. whole saliva (approximately 2 ml) was collected into sterile 50 ml polypropylene tubes (Costar), and centrifuged for 5 min at 1300×*g*. The resulting pellet was resuspended in 200 μl of Binding buffer and forty microliters of Proteinase K were added. The samples were mixed thoroughly and immediately incubated for 10 min at 70°C, followed by addition of 100 μl of isopropanol and mixed well. The lysate was subsequently applied to a spin column with a silica gel-based membrane for micro spin purification (provided in the kit) and spin at 8000×*g*, 1 min at room temperature. Then 500 μl of Inhibitor Removal Buffer were added to the upper spin column and centrifuged at 8,000xg 1 min at room temperature. The spin column was washed twice (8000×*g*, 1 min, room temperature) with 500 μl Wash buffer. In order to elute membrane-bound DNA, 200 μl of Elution buffer (preheated to 70°C) were added to the column and incubated at 70°C for 5 min. The column was thereafter centrifuged (8000×*g*, 1 min, room temperature) and the eluate was stored at minus 20°C until analysis. Binding buffer, Proteinase K, Inhibitor Removal Buffer, Wash buffer and Elution buffer were provided in the kit. The DNA isolated from whole saliva using a commercially available isolation kit is a suitable alternative to DNA isolated from blood when employed as a template for PCR-based analysis of biallelic polymorphisms (van Schie *et al.*, 1997). The advantage using saliva for DNA isolation is that its collection is fast, easy, inexpensive and non-invasive.

Genotyping of *IL-4* gene polymorphisms

The *IL-4* -590 C/T (rs2243250) and -33 C/T (rs2070874) single nucleotide polymorphisms (SNPs) were investigated using PCR-restriction fragment length polymorphism (RFLP) analysis as described previously with some modification (Gyan *et al.*, 2004; Lu *et al.*, 2014). Briefly, the PCR amplification was performed in 10 μl reactions using 1 μl of genomic DNA, 5 μl of 2× ready-to-use PCR Master Mix (iNtRON biotechnology, Korea) and 5 pmoles of each primer. The PCR was carried out in an Eppendorf (Corning, USA) using a 2 min denaturation at 94°C followed by 35 cycles with 94°C for 20 sec, 62°C for 10 sec and 72°C for 30 sec. The final extension was at 72°C for 5 min. After amplification, the PCR products were then digested with *BsmF1* and *BsmA1* restriction enzyme (New England Biolabs, UK) for *IL-4*-590 C/T and

IL-4-33 C/T respectively. The digested products were analyzed on 2.5% agarose gel containing ethidium bromide. The following fragments were obtained : *IL-4*-590: C/C 192 and 60 bp, T/T 252 bp; *IL-4*-33: T/T 144, 38, 129 bp, CC 182 129. The samples were test in duplicate by different persons and the results were complete concordance.

IL-4 VNTR intron 3 (rs79071878) were PCR amplified as described previously with some modification (Birbian *et al.*, 2014). Briefly, the PCR was carried out in a total volume of 10 μl containing 1 μl of genomic DNA, 5 μl of 2× ready-to-use PCR Master Mix (iNtRON biotechnology, Korea) and 5 pmoles of each primer. The PCR conditions were : initial denaturation at 94°C for 2 min, followed by 35 cycles with 94°C for 20 sec, 63°C for 10 sec and 72°C for 30 sec. The final extension was at 72°C for 5 min. After amplification, the PCR products were analyzed directly on 2% agarose gel containing ethidium bromide and visualized by UV transillumination. The size of the product corresponds to the number of the repeats present (70 bp repeat). A 254 bp product (three repeats) indicated the presence of homozygous wild R3/R3 genotype while a 184 bp product (two repeats) marked the presence of the homozygous mutant R2/R2 genotype. The presence of 184 bp and 254 bp products indicated the heterozygous R2/R3 genotype. A 114 bp product (a single repeat : R1) was rarely occurring. However, the heterozygous R2/R1 genotype was present in our set. Similar to the genotyping of *IL-4* -590 C/T and -33 C/T, the genotyping of *IL-4* VNTR intron 3 was done in duplicate by different persons and the results were complete concordance.

Statistical analysis

Allele and genotype distribution of the *IL-4* -590 C/T, -33 C/T, VNTR intron 3 polymorphisms between PTB patients and HCS were analyzed statistically using the Chi-square ($\chi 2$) test. The data was analyzed using SPSS software. Fisher's exact test was used wherever applicable. A *p*-value of 0.05 or less was considered to be significant, and *p*-values between 0.05 – 0.1 were considered to be marginally significant.

RESULTS

The distribution of genotype and allele frequencies of three investigated *IL-4* polymorphisms (*IL-4* -590 C/T, -33 C/T, *IL-4* VNTR 70bp intron 3) in Thai pulmonary tuberculosis (PTB) patients and healthy control subjects (HCS) were summarized in Table 1. All of the three investigated loci in the *IL-4* gene were in Hardy-Weinberg equilibrium ($p>0.05$, data not shown). Neither the allele nor the genotype frequencies of *IL-4* polymorphisms in this study were significant differences between the PTB patients and healthy controls (P>0.05). However, the allele distribution of the *IL-4* -590 C, -33 C, and VNTR R3 was higher among PTB patients (25.58%, 25.58%, 25.58%, respectively) than among control subjects (20%, 20.48%, 19.44%, respectively); whereas the *IL-4* -590 T, -33 T, and VNTR R2 allele was more prevalent among healthy control subjects (80%, 79.52%, 80%, respectively) than among PTB patients (74.42%, 74.42%, 74.42%, respectively).

In this study, linkage disequilibrium was observed among *IL-4* -590 C/T, -33 C/T, and *IL-4* VNTR 70bp intron 3 polymorphisms. Mostly, the homozygous of *IL-4* -590 TT, -33 TT, and VNTR R2R2 was represented together, while the heterozygous of CT, CT, and R2R3 or homozygous of CC, CC, R3R3 was represented together. Complete linkage disequilibrium between *IL-4* -590 C/T and *IL-4* -33C/T polymorphisms was observed in both PTB and control subjects groups. For the *IL-4* VNTR, two of 43 in PTB patients and 4 of 83 in healthy control subjects did not showed those completed linkage disequilibrium with the *IL-4* -590 C/T and *IL-4* -33C/T polymorphisms. In haplotype analysis, five haplotypes were detected: TTR2, CCR3, CCR2, TTR3, and TTR1. When haplotype frequencies were compared between PTB and HCS, similar results were obtained with the allele frequencies results. The TTR2

Table 1. Distribution of genotype and allele frequencies (*IL-4* -590 C/T rs2243250, -33 C/T rs2070874, *IL-4* variable number of tandem repeats [VNTR] 70bp deletion intron 3 rs79071878) between Thais pulmonary tuberculosis (PTB) patients and healthy control subjects (HCS)

IL-4 polymorphisms	PTB N = 43(%)	HCS N = 90(%)	PTB vs HCS	
			p-value (a)	OR (95% CI)
-590 T/C				
TT	25 (58.14)	58 (64.44)	0.88	
CT	14 (32.56)	28 (31.11)		
CC	4 (9.30)	4 (4.44)		
Allele				
T	64 (74.42)	144 (80)	0.38	1.38 (0.75-2.52)
C	22 (25.58)	36 (20)		
TT vs CT+CC	25 vs 18	58 vs 32	0.61	1.31 (0.62-2.75)
CC vs CT+TT	4 vs 39	4 vs 86	0.47	0.45 (0.11-1.91)
-33 T/C				
TT	25 (58.14)	53 (63.86)	0.92	
CT	14 (32.56)	26 (31.33)		
CC	4 (9.30)	4 (4.82)		
Allele				
T	64 (74.42)	132 (79.52)	0.44	1.33 (0.72-2.46)
C	22 (25.58)	34 (20.48)		
TT vs CT+CC	25 vs 18	53 vs 30	0.66	1.27 (0.60-2.70)
CC vs CT+TT	4 vs 39	4 vs 79	0.55	0.49 (0.12-2.08)
70bp VNTR				
R2R1	0	1 (1.11)	0.40	
R2R2	25 (58.14)	58 (64.44)		
R2R3	14 (32.56)	27 (30.00)		
R3R3	4 (9.30)	4 (4.44)		
Allele				
R1	0	1 (0.56)	0.53	
R2	64 (74.42)	144 (80.00)		
R3	22 (25.58)	35 (19.44)		
R2R2 vs R2R1+R2R3+R3R3	25 vs 18	58 vs 32	0.61	1.31 (0.62-2.75)
R3R3 vs R2R1+R2R3+R2R2	4 vs 39	4 vs 86	0.47	0.45 (0.11-1.91)

(a) Chi-square test or Fisher's exact test, OR = odds ratio, CI = confidence interval.

haplotype frequencies in PTB were lower than in HCS (73.25% vs 78.31%), while CCR3 haplotype frequencies in PTB were higher than in HCS (24.42% vs 19.27%). Table 2.

The distributions of *IL-4* -590 C/T and -33 C/T in healthy control subjects in this study were compared to the available allele frequencies in world populations as observed in Hapmap database (Table 3) (NCBI-Hapmap, 2018). It was revealed that the allele frequencies of *IL-4* -590 C/T and -33 C/T in healthy control subjects in this study were accordance with those allele frequencies reported in Chinese and Japanese population.

DISCUSSION

All of the three investigated *IL-4* polymorphisms in this study including -590 C/T, -33 C/T, and VNTR intron 3 were not significantly associated with pulmonary tuberculosis (PTB) in Thai people living in the central area of Thailand. In addition, we found that the *IL-4* VNTR 70 bp intron 3 allele R1 with a single tandem repeat was present in 0.56% of the Thai population studied.

IL-4, a key regulator of Th2-type responses, plays an essential role in maintaining balance in the anti-microbial

Table 2. Distribution of *IL-4* -590 C/T rs2243250, -33 C/T rs2070874, *IL-4* variable number of tandem repeats [VNTR] 70bp deletion intron 3 rs79071878: The genotype and haplotype frequencies between pulmonary tuberculosis (PTB) patients and healthy control subjects (HCS)

IL-4-590/-33/VNTR intron 3	PTB	HCS
Genotype	Genotype frequencies (%)	
TT/TT/R2R2:TTR2/TTR2	25 (58.14)	51 (61.45)
CT/CT/R2R3:CCR3/TTR2	13 (30.23)	24 (28.92)
CC/CC/R3R3:CCR3/CCR3	3 (7.98)	4 (4.82)
CC/CC/R2R3:CCR2/CCR3	1 (2.33)	0
CT/CT/R3R3:CCR3/TTR3	1 (2.33)	0
CT/CT/R2R2:CCR2/TTR2	0	2 (2.41)
TT/TT/R2R1:TTR2/TTR1	0	1 (1.20)
TT/TT/R2R3:TTR2/TTR3	0	1 (1.20)
Haplotypes	Haplotype frequencies (%)	
TTR2	63 (73.25)	130 (78.31)
CCR3	21 (24.42)	32 (19.27)
CCR2	1 (1.16)	2 (1.20)
TTR3	1 (1.16)	1 (0.60)
TTR1	0	1 (0.60)

Table 3. Observed allele frequencies of Thais healthy control subjects in the current study compared to reported allele frequencies in world populations

HAPMAP populations (Hapmap database)	Rs2243250 (-590C/T)		rs2070874 (-34C/T)	
	C	T	C	T
Thais – current study	0.20	0.80	0.20	0.80
African ancestry in Southwest USA (ASW)	0.43	0.57	0.60	0.40
Han-Chinese in Beijing (CHB)	0.26	0.74	0.26	0.74
Southern Han Chinese (CHS)	0.20	0.80	0.20	0.80
Gujarati Indians in Houston, Texas (GIH)	0.89	0.11	0.89	0.11
Japanese in Tokyo (JPT)	0.27	0.73	0.27	0.73
Luhya in Webuye, Kenya (LWK)	0.19	0.81	0.42	0.58
Toscans in Italy (TSI)	0.88	0.12	0.87	0.13

response of the immune system. The functional roles of *IL-4* polymorphisms have been well documented in many diseases, including PTB. Our study did not observe any significant differences either in the allele or genotype frequencies of *IL-4* -590 C/T, -33 C/T, and VNTR intron3 between PTB patients and healthy controls. These findings consistent with those studies in Chinese, Brazilian and Turkish populations where there were no association between *IL-4* -590 C/T SNP and TB (Qi *et al.*, 2014; Milano *et al.*, 2016; Ulger *et al.*, 2013). The case-control base association in this study and the family base association test of single nucleotide polymorphism (SNP) in chromosome 5q31 gene in TB patients in the north of Thailand were compared. Similar results were obtained, the family base association test in the north did not reveal any noticeable association between *IL-4* SNP and TB (Ridruechai *et al.*, 2010). Thus, people susceptible to TB living in the north and central area of Thailand may have similar genetic background. Although, the current study did not observe a significant association between *IL-4* -590 C/T, -33 C/T, and VNTR intron3 and PTB, the allele distribution

of the *IL-4* -590 C, -33 C, and VNTR R3 was higher among PTB patients (25.58%, 25.58%, 25.58%, respectively) than among control subjects (20%, 20.48%, 19.44%, respectively). Accordingly, the results demonstrating a higher frequency of *IL-4* -590 C allele in this study were similar to the studies in Iranian, Russian and Indian population, where the C allele was significantly increased in TB patients when compared to controls (Amirzargar *et al.*, 2006; Naslednikova *et al.*, 2009; Sivangala *et al.*, 2014).

In the current study, the allele frequencies of *IL-4* -590 C/T and -33 C/T in healthy control subjects and PTB patients were in accordance with the frequencies reported in Chinese and Japanese population deposited in the Hapmap database (Table 3.) (NCBI-Hapmap, 2018). A study by the Japanese group explored an association among *IL-4* -590 C/T, -33 C/T and VNTR intron 3 and found strong linkage disequilibrium among these SNPs and VNTR. Their results indicated that there are only two haplotypes in the Japanese population: -590T, -33T and VNTR R2 (two repeat) are on haplotype I, while -590C, -33C and VNTR R3 (three repeat) are on the other (haplotype II) (Nakashima *et al.*, 2002). Similar results were observed in the Thai population, where strong linkage disequilibrium among -590C/T, -33C/T and VNTR intron 3 were showed in this study. However, two of 43 in PTB patients and 4 of 83 in healthy control subjects did not showed completed linkage disequilibrium between the *IL-4* -590 C/T-*IL-4* -33 C/T and *IL-4* VNTR intron 3 polymorphisms. Thus five haplotypes were observed in this study : TTR2, CCR3, CCR2, TTR3, and TTR1. Jha *et al.* reported that mainly two and three copies of a 70bp repeat (VNTR intron 3) have been observed in humans and were designated as R2 and R3 respectively; whereas only a single copy of 70 bp repeat (R1;114bp) had been observed in other primates (Jha *et al.*, 2012). However, a heterozygous of *IL-4* VNTR R2R1 genotype was present in HCS in this study at a frequency of 1.11%.

In conclusion, *IL-4* -590 C/T, *IL-4* -33 C/T and *IL-4* VNTR intron 3 polymorphisms in this study did not show a significant association with PTB susceptibility in the Thai population living in the central area of Thailand. Although no significant associations between *IL-4* -590 C/T, -33 C/T, and VNTR intron 3 and PTB were observed, the allele distribution of the *IL-4* -590 C, -33 C, and VNTR R3 was higher among PTB patients than among control subjects. This may indicate that *IL-4* -590 C/T, -33 C/T and VNTR intron 3 might play a role in susceptibility to PTB. However, a larger cohort may further confirm and support the findings in this study.

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DISCLOSURE

The authors declare having no conflict of interest.

REFERENCES

Amirzargar, A.A., Rezaei, N., Jabbari, H., Danesh, A.A., Khosravi, F., Hajabdolbaghi, M., Yalda, A. & Nikbin, B. (2006). Cytokine single nucleotide polymorphisms in Iranian patients with pulmonary tuberculosis. *European Cytokine Network* **17**: 84-89.

Arend, S.M., Engelhard, A.C., Groot, G., de Boer, K., Andersen, P., Ottenhoff, T.H. & van Dissel, J.T. (2001). Tuberculin skin testing compared with T-cell responses to Mycobacterium tuberculosis-specific and nonspecific antigens for detection of latent infection in persons with recent tuberculosis contact. *Clinical and Diagnostic Laboratory Immunology* **8**: 1089-1096.

Birbian, N., Singh, J., Jindal, S.K. & Sobti, R.C. (2014). High risk association of IL-4 VNTR polymorphism with asthma in a North Indian population. *Cytokine* **66**: 87-94.

Dlugovitzky, D., Torres-Morales, A., Rateni, L., Farroni, M.A., Largacha, C., Molteni, O. & Bottasso, O. (1997). Circulating profile of Th1 and Th2 cytokines in tuberculosis patients with different degrees of pulmonary involvement. *FEMS Immunology and Medical Microbiology* **18**: 203-207.

Durie, F.H., Foy, T.M., Masters, S.R., Laman, J.D. & Noelle, R.J. (1994). The role of CD40 in the regulation of humoral and cell-mediated immunity. *Immunology Today* **15**: 406-411.

Gyan, B.A., Goka, B., Cvetkovic, J.T., Kurtzhals, J.L., Adabayeri, V., Perlmann, H., Lefvert, A.K., Akanmori, B.D. & Troye-Blomberg, M. (2004). Allelic polymorphisms in the repeat and promoter regions of the interleukin-4 gene and malaria severity in Ghanaian children. *Clinical and Experimental Immunology* **138**: 145-150.

Hasan, Z., Jamil, B., Khan, J., Ali, R., Khan, M.A., Nasir, N., Yusuf, M.S., Jamil, S., Irfan, M. & Hussain, R. (2009). Relationship between circulating levels of IFN-gamma, IL-10, CXCL9 and CCL2 in pulmonary and extrapulmonary tuberculosis is dependent on disease severity. *Scandinavian Journal of Immunol*ogy **69**: 259-267.

Hill, A.V. (2001). The genomics and genetics of human infectious disease susceptibility. *Annual Review of Genomics Human Genetics* **2**: 373-400.

Jeronimo, S.M., Holst, A.K., Jamieson, S.E., Francis, R., Martins, D.R., Bezerra, F.L., Ettinger, N.A., Nascimento, E.T., Monteiro, G.R., Lacerda, H.G., Miller, E.N., Cordell, H.J., Duggal, P., Beaty, T.H., Blackwell, J.M. & Wilson, M.E. (2007). Genes at human chromosome 5q31.1 regulate delayed-type hypersensitivity responses associated with Leishmania chagasi infection. *Genes and Immunity* **8**: 539-551.

Jha, AN1, S.V., Kumari, N., Singh, A., Antony, J., van Tong, H., Singh, S., Pati, S.S., Patra, P.K., Singh, R., Toan, N.L., Song, le H., Assaf, A., Messias-Reason, I.J., Velavan, T.P., Singh, L. & Thangaraj, K. (2012). IL-4 haplotype -590T, -34T and intron-3 VNTR R2 is associated with reduced malaria risk among ancestral indian tribal populations. *PLoS One* **7**: e48136.

Jiang, H., Harris, M.B. & Rothman, P. (2000). IL-4/IL-13 signaling beyond JAK/STAT. *Journal of Allergy and Clinical Immunol*ogy **105**: 1063-1070.

Kahnert, A., Seiler, P., Stein, M., Bandermann, S., Hahnke, K., Mollenkopf, H. & Kaufmann, S.H. (2006). Alternative activation deprives macrophages of a coordinated defense program to Mycobacterium tuberculosis. *European Journal of Immunology* **36**: 631-647.

Kulpraneet, M., Limtrakul, A., Srisurapanon, S. & Tangteerawatana, P. (2015). Lack of Association between IL-10 Gene Promoter Polymorphisms and Susceptible to Tuberculosis in Thai Patients. *Journal of the Medical Association of Thailand* 98 Suppl **10**: S124-129.

Lu, Y., Wu, Z., Peng, Q., Ma, L., Zhang, X., Zhao, J., Qin, X. & Li, S. (2014). Role of IL-4 gene polymorphisms in HBV-related hepatocellular carcinoma in a Chinese population. *PLoS One* **9**: e110061.

Luoni, G1., Verra, F., Arcà, B., Sirima, B.S., Troye-Blomberg, M., Coluzzi, M., Kwiatkowski, D. & Modiano, D. (1995). Antimalarial antibody levels and IL-4 polymorphism in the Fulani of West Africa. *Genes and Immunity* **2**: 411-414.

Marsh, D.G., Neely, J.D., Breazeale, D.R., Ghosh, B., Freidhoff, L.R., Ehrlich-Kautzky, E., Schou, C., Krishnaswamy, G. & Beaty, T.H. (1994). Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* **264**: 1152-1156.

Milano, M., Moraes, M.O., Rodenbusch, R., Carvalho, C.X., Delcroix, M., Mousquer, G., Laux da Costa, L., Unis, G., Dalla Costa, E.R. & Rossetti, M.L. (2016). Single Nucleotide Polymorphisms in IL17A and IL6 Are Associated with Decreased Risk for Pulmonary Tuberculosis in Southern Brazilian Population. *PLoS One* **11**: e0147814.

Moller, M. & Hoal, E.G. (2010). Current findings, challenges and novel approaches in human genetic susceptibility to

tuberculosis. *Tuberculosis (Edinb)* **90**: 71-83.

Nakashima, H., Miyake, K., Inoue, Y., Shimizu, S., Akahoshi, M., Tanaka, Y., Otsuka, T. & Harada, M. (2002). Association between IL-4 genotype and IL-4 production in the Japanese population. *Genes and Immunity* **3**: 107-109.

Naslednikova, I.O., Urazova, O.I., Voronkova, O.V., Strelis, A.K., Novitsky, V.V., Nikulina, E.L., Hasanova, R.R., Kononova, T.E., Serebryakova, V.A., Vasileva, O.A., Suhalentseva, N.A., Churina, E.G., Kolosova, A.E. & Fedorovich, T.V. (2009). Allelic polymorphism of cytokine genes during pulmonary tuberculosis. *Bulletin of Experimental Biology and Medicine* **148**: 175-180.

NCBI (The National Center for Biotechnology Information). Hapmap. 25 november 2018. Available from: https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/.

Qi, H., Sun, L., Jin, Y.Q., Shen, C., Chu, P., Wang, S.F., Yin, Q.Q., Qi, Z., Xu, F., Jiao, W.W., Wu, X.R., Tian, J.L., Xiao, J. & Shen, A.D. (2014). rs2243268 rs2243274 of Interleukin-4 (IL-4) gene are associated with reduced risk for extrapulmonary and severe tuberculosis in Chinese Han children. *Infection*, *Genetic and Evolution* **23**: 121-128.

Ridruechai, C., Mahasirimongkol, S., Phromjai, J., Yanai, H., Nishida, N., Matsushita, I., Ohashi, J., Yamada, N., Moolphate, S., Summanapan, S., Chuchottaworn, C., Manosuthi, W., Kantipong, P., Kanitvittaya, S., Sawanpanyalert, P., Keicho, N., Khusmith, S. & Tokunaga, K. (2010). Association analysis of susceptibility candidate region on chromosome 5q31 for tuberculosis. *Genes and Immunity* **11**: 416-422.

Rosenwasser, L.J., Klemm, D.J., Dresback, J.K., Inamura, H., Mascali, J.J., Klinnert, M. & Borish, L. (1995). Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. *Clinical and Experimental Allergy* **25** Suppl 2: 74-78.

Selvaraj, P., Alagarasu, K., Harishankar, M., Vidyarani, M., Nisha, Rajeswari, D. & Narayanan, P.R. (2008). Cytokine gene polymorphisms and cytokine levels in pulmonary tuberculosis. *Cytokine* **43**: 26-33.

Sivangala, R., Ponnana, M., Thada, S., Joshi, L., Ansari, S., Hussain, H., Valluri, V. & Gaddam, S. (2014). Association of cytokine gene polymorphisms in patients with tuberculosis and their household contacts. *Scandinavian Journal of Immunology* **79**: 197-205.

Ulger, M., Emekdas, G., Aslan, G., Tas, D., Ilvan, A., Tezcan, S., Calikoglu, M., Erdal, M.E. & Kartaloglu, Z. (2013). Determination of the cytokine gene polymorphism and genetic susceptibility in tuberculosis patients. *Mikrobiyoloji Bulteni* **47**: 250-264.

van Schie, R.C. & Wilson, M.E. (1997). Saliva: a convenient source of DNA for analysis of bi-allelic polymorphisms of Fc gamma receptor IIA (CD32) and Fc gamma receptor IIIB (CD16). *Journal of Immunological Methods* **208**: 91-101.

Vannberg, F.O., Chapman, S.J., Hill, A.V. (2011). Human genetic susceptibility to intracellular pathogens. *Immunology Review* **240**: 105-116.

Vidyarani, M., Selvaraj, P., Prabhu Anand, S., Jawahar, M.S., Adhilakshmi, A.R. & Narayanan, P.R. (2006). Interferon gamma (IFNgamma) & interleukin-4 (IL-4) gene variants & cytokine levels in pulmonary tuberculosis. *Indian Journal of Medical Research* **124**: 403-410.

World Health Oganization. GLOBAL TUBERCULOSIS REPORT 2017: Executive Summary. 4 Decenber 2017. Available from: http://www.who.int/tb/publications/global_report/Exec_Summary_13Nov2017.pdf?ua=1.

World Health Oganization. Tuberculosis (TB). 4 Decenber 2018. Available from: http://www.searo.who.int/thailand/areas/tuberculosis/en/.