Relationship Between TNF-238G>A Polymorphism and Predisposition to Pulmonary Tuberculois Infection in The Indonesian Population (A Pilot Study)

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ABSTRACT *Previous studies suggested that genetic factors exerted huge influence in susceptibility to Tuberculosis. Tumor Necrosis Factor Alpha (TNF-a), which is encoded by the TNF gene, play a role on pulmonary macrophage function in isolating and controlling Mycobacterium tuberculosis infection. Polymorphisms on the promoter region of the TNF gene have been predicted to affect its transcriptional activity. Therefore, these polymorphisms are an excellent candidate to further study the role of TNF-a in susceptibility to Tuberculosis. 100 pulmonary tuberculosis patients (case) and 100 healthy individuals (controls) were recruited for this pilot study. DNA samples from cases and controls were genotyped for the TNF -238G>A SNP (rs361525) using LightSNip genotyping assay. Our results showed no significant difference in the distribution of TNF -238 genotypes in case and control subjects (P = 0.4335). Further investigation on TNF -238 allele frequencies between case and control studies also yields no significant difference (P=1.000; OR=1.000; %95CI [0.246597 – 4.055200]) which may suggest that there are no association with predisposition to Tuberculosis infection. In conclusion, this pilot study showed that the TNF -238G>A SNP is not associated with susceptibility to Tuberculosis.*

BACKGROUND

Tuberculosis (TB) is a fatal but curable disease, resulting from pulmonary alveolar infection by *Mycobacterium tuberculosis* (MTB). Despite the implementation of the DOTS (Directly Observed Treatment Short-course) strategy since 1994, Indonesia currently still has the fifth largest TB incidence in the world (WHO 2014). TB is known to be contagious, and is still responsible for high mortality worldwide (WHO 2014). However, it is reported that only 10% of people infected with MTB progress to active TB disease (Bellamy 1998; Kleinnijenhuis *et al.* 2011; Murray, Styblo & Rouillon 1990).

Observations on this disease in several human populations with different race and ethnicities, showed higher TB infection in certain populations (Motulsky 1989; Stead *et al*. 1990). This suggests that genetic factors played a role in susceptibility to TB infection. Twin studies have also been conducted, which further demonstrates the genetic nature of susceptibility to TB (Comstock 1978; Kallmann & Reisner 1943). However, the exact genetic factors or molecular mechanisms involved, to date, remains elusive.

Single nucleotide polymorphisms (SNPs) are known to be the most prevalent genetic variations found among humans. Several candidate genes which have known biological function related to TB infection in humans such as HLA (Human Leucocyte Antigen), TLR (Toll-like receptor), CD209 (C-type lectin DC-SIGN) and IRF1 (Interferon Regulatory Factor 1), have been investigated and SNPs found in these genes showed association with susceptibility or protection to TB (Ding *et al*. 2012; Kim *et al*. 2005; Kobayashi *et al*. 2012; Vannberg *et al*. 2008). Many of the genes found to significantly affect susceptibility to TB played a role in immune regulation pathways. Thus host genetic factors, which participated in the host immune response, are excellent candidates to study and further understand genetic susceptibility to TB.

The pro-inflammatory cytokine Tumor Necrosis Factor Alpha (TNF-α), encoded by the *TNF* gene, is produced mainly by macrophages and other white blood cells derived from monocytes. Mechanistic studies have shown that TNF-α play a huge role in MTB infection particularly in macrophage activation, recruitment to site of infection and granuloma formation which contains and controls the disease (Flesch & Kaufmann 1990; Flynn *et al*. 1995; Lin *et al*. 2007; Mohan *et al*. 2001). Moreover, a study in rheumatoid arthritis patients treated with the Anti-TNF drug Infliximab showed that patients with latent TB progress to active TB following treatment with Infliximab (Keane *et al*. 2001).

Thus, it is postulated that the regulation of TNF-α might affect susceptibility to TB. Single nucleotide polymorphisms (SNP) found on the promoter region of the TNF gene, such as rs361525 (TNF -238) and rs1800629 (TNF -308), have been vastly studied (Abraham & Kroeger 1999).

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Polymorphisms on promoter region of the TNF gene were predicted to influence its transcriptional activity (Goldfeld, Doyle & Maniatis 1990). In relation to TB susceptibility, TNF -238 polymorphism have been showed to increase the risk of active TB (Amirzargar *et al*. 2006; Correa *et al*. 2005; Oliveira *et al*. 2004).

Others have also reported that polymorphisms in the promoter region of TNF have been associated with susceptibility or increased severity to other infectious agents such as Hepatitis B virus, A/H1N1 virus, Malaria parasite and *Helicobacter pylori* (Flori *et al*. 2005; Höhler *et al*. 1998; Morales-Garcia *et al*. 2012; Yea *et al*. 2001). Therefore, we proposed that certain TNF promoter genetic variants, might affect susceptibility to TB, specifically in the Indonesian population. The study of disease susceptibility genes will allow us to understand better the infection mechanism of TB which in turn will allow us to devise a better strategy for TB disease prevention and eradication.

METHODS

Study Participants

100 TB patients and 100 healthy individual were recruited for the study. All patients and controls are of Indonesian background with Javanese and Sundanese ethnicity, sex and age. Active TB patients were confirmed by Acid-Fast Bacilli smear test. All study participants had signed an informed consent form and this study was approved by the Universitas YARSI Research Ethics Committee.

SNP genotyping

DNA were extracted using the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Extracted DNA were quantified using a Qubit 2.0 Fluorometer (Invitrogen, California, USA) prior to SNP genotyping. Genotyping of the TNF -238 SNP were accomplished using LightSNiP rs361525 TNF G-238A genotyping assay based on the SimpleProbe probe genotyping system (TIB MOLBIOL, Berlin, Germany) on a LightCycler 480 II real-time PCR instrument (Roche Applied Science, Mannheim, Germany). Realtime PCR was performed in a 5 µL reaction mixture containing approximately 10 ng of genomic DNA, 1X of 10X Lightcycler FastStart DNA Master Hybprobe, 1X of 20X LightSNiP reagent mix (primers and probes mix) and 3 mM $MgCl_2$, using 384-well plates. The PCR conditions are as follow: initial denaturation at 96°C for 4 minutes; 40 cycles of denaturation at 96°C for 30 seconds, annealing at 55 °C for 30 seconds, and elongation at 72°C for 30 seconds; and a final elongation step at 72°C for 5 minutes.

Data Interpretation and Statistical Analysis

Due to the unavailability of control samples with known genotypes, the LightSNip assay results, particularly from control samples, were calibrated with the genetic frequency of rs361525 from public genetic population data on dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=361525). This is our method to define unknown arbitrary genotypes used in the assay. X^2 test and Fisher exact test were used to assess correlation between genotypes or alleles and phenotypes of both cases and controls, where appropriate. P value of <0.05 was considered

statistically significant. Odd ratios estimate relative risk with 95% confidence with. GraphPad Prism Software version 5.06 (GraphPad, San Diego, USA) was used for all statistical calculations.

RESULTS

The LightSNip assay was assessed by melting analysis which is shown in figure 1. Each distinct melting curve represents 1 sample and each melting peaks represent different alleles/genotypes. As the required known genotype controls were not available, arbitrary genotypes AA, AB and BB, were used to define the homozygous and heterozygous melting peaks. The genotype frequencies of for the healthy controls are 96%, 4% and 0% for AA, AB and BB respectively. According to the public SNP database on TNF-238, G (Guanine) is the frequent allele and A (Adenine) is the rare allele, particularly in Asian populations. Thus, we can assume that the arbitrary genotypes AA, AB and BB can be translated to GG, GA and AA genotypes respectively.

As shown in Table 1, frequency of the homozygous GG genotype was relatively similar between TB patients (cases) and healthy individuals (controls) which were 97% and 96% respectively. The heterozygous GA genotype was present at 2% and 4% for cases and controls respectively. However, the homozygous AA genotype was found only in TB patients. Assessment of Hardy-Weinberg Equilibrium showed that population of the control group does not deviate from Hardy-Weinberg proportions (p=0.838298).

Statistical analysis between genotype frequencies of cases and controls showed no significant differences between groups (P = 0.4335). TNF-238 allele frequencies between cases and controls were shown in Table 2. No significant differences in allele frequencies were found between case and control group (P=1.000; OR=1.000; %95CI [0.246597 – 4.055200]). The curve peaks at the left hand side stand for the arbitrary A allele and at the right hand side (red) stands for the B allele. Single peaks represent homozygotes (AA - Blue or BB - green) while double peaks represent heterozygotes (AB - red). Due to unavailability of known genotype controls, arbitrary genotypes were used and correlation with public SNP population database was utilized to determine true genotypes.

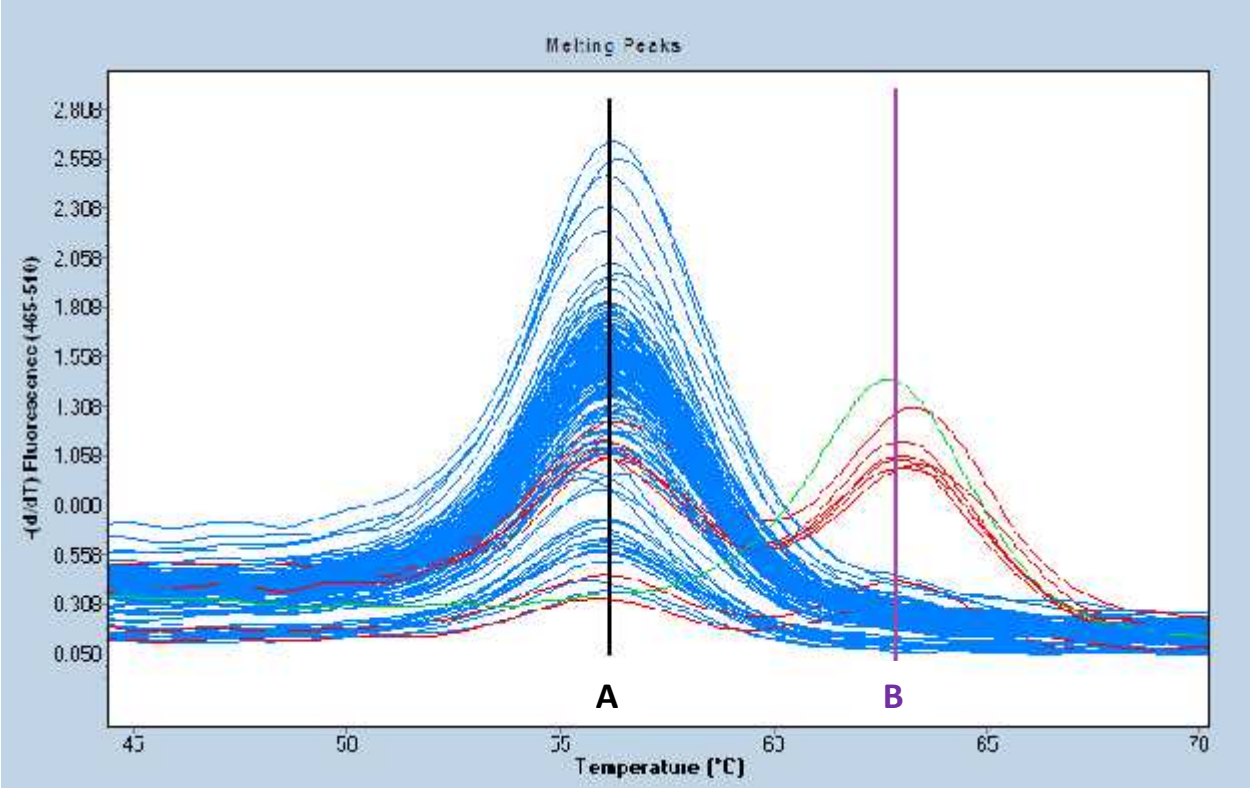

Figure 1. Melting analysis to determine TNF -238 genotypes in cases and controls.

Table 1. Genotype frequencies of the TNF -238 SNP between cases (TB patients) and controls (Healthy individuals)

Genotype	TB patients [*n* (%)]	Controls [*n* (%)]
GG	97 (97)	96 (96)
GA	2 (2)	4 (4)
AA	1 (1)	0 (0)
P = 0.4335		

Table 2. Allele frequencies of the TNF -238 SNP between cases (TB patients) and Controls (Healthy Individuals)

Allele	TB patients [*n* (%)]	Controls [*n* (%)]
G	196(98)	196(98)
A	4(2)	4(2)
P=1.000; OR=1.000; %95CI [0.246597 – 4.055200]		

DISCUSSION

Our current data showed no association between TNF -238 genotype (P = 0.4335) and allele (P = 1.000) variants with susceptibility to TB. Thus, our study contradicts the reports by Oliveira *et al*. in the Brazilian population, Correa *et al*. in the Northwestern Colombian (Spanish) population and Amirzargar *et al*. in the Iranian population at the same SNP position (Amirzargar *et al*. 2006; Correa *et al*. 2005; Oliveira *et al*. 2004). In spite of these inconsistencies with the previously mentioned studies, there appears to be abundant reports which suggests that TNF-238 alone does not correlate well with increased risk of susceptibility to TB (Anoosheh, Farnia & Kargar 2011; Ates *et al*. 2008; Selvaraj *et al*. 2001; Vejbaesya *et al*. 2007). TNF promoter polymorphism was known to be ethnic-specific (Baena *et al*. 2002). In terms of ethnicity, the previous studies by Anoosheh *et al*., Selvaraj *et al.* Ates *et al* and Vejbaesya *et al*, were conducted in the Iranian, Indian, Turkish and Thai populations respectively. Therefore, the positive studies by Amizargar *et al*., Oliveira *et al*. and Correa *et al*. may not correspond well with our study, due to differences in population genetic backgrounds. The study by Vejbaesya *et al*., which was done in the Thai population, was presumed to be the closest to us in terms of ethnicity and genetic background (Hatin *et al*. 2014).

A study in The Cambodian population showed that the TNF -238A allele was quite rare in that specific population, with frequency below 2% in healthy individuals (Delgado *et al.* 2002). Our study showed that the A allele was at most 2% in the population, as shown in the controls, which was consistent with the study in the Cambodian population. Furthermore, TNF -238A frequency in the Thai population from the study by Vejbaesya *et al*. was quite close (3.4%) with the study in the Cambodian population. On the other hand, the study in the Colombian population showed that the frequency of the TNF-238A allele in healthy individuals was much higher (11%) than both Cambodia and Thailand (Henao *et al*. 2006). Therefore, these studies highlight the differences in TNF promoter polymorphisms frequencies between populations, which further support the result of this study.

The influence of TNF promoter polymorphisms on transcriptional activity of TNF polymorphism is currently a controversial topic in the field. There are 10 SNPs that have been discovered in the promoter region of the TNF-gene: -1031T>C, -863C>A, -

857C>A, -851C>T, -419G>C, -376G>A, -308G>A, -238G>A,-162G>A and -49G>A. Several TNF promoter polymorphisms have been studied regarding their effect on TNF transcriptional activity and gene expression. Among those TNF promoter polymorphisms, TNF -308G>A is the most well studied (Bayley, Ottenhoff & Verweij 2004). A study by Wilson *et al*. in human B cell line showed that the A allele of TNF -308 had higher transcriptional activity compared to the G allele (Wilson *et al*. 1994). Studies by Kroeger *et al*. and Abraham *et al*. confirmed the previous study, which showed that TNF -308 influenced TNF gene expression (Abraham & Kroeger 1999; Kroeger, Carville & Abraham 1997). However, others have not been able to duplicate the previous results (Brinkman *et al*. 1995; Knight *et al*. 2003; Mekinian *et al*. 2011).

On the other hand, several studies investigating the functional significance of TNF -238G>A have not found concrete evidence related to increased or decreased TNF promoter transcriptional activity or gene expression (Huizinga *et al*. 1997; Kaijzel *et al*. 1998; Pociot *et al.* 1995; Uglialoro *et al.* 1998). Nonetheless, several studies in various infectious diseases have shown the importance of TNF -238 G>A in disease susceptibility, albeit with currently unknown molecular mechanism. In another study, Skoog *et al.* showed evidence that TNF -863C>A reduced TNF plasma levels and transcriptional activity of TNF by 31% (Skoog *et al*. 1999). Moreover, Higuchi *et al*. also showed increased TNF transcriptional activity in individuals of Japanese descent with TNF -1031C and -863A alleles (Higuchi *et al*. 1998). However, no known study have shown significant associations between these SNPs with susceptibility to Tuberculosis. Elahi *et al*. have hypothesized that due to the abundance of SNPs in the promoter region of TNF, other SNPs in the coding region of TNF might have higher functional significance in relation to TNF gene function (Elahi *et al.* 2009). Therefore, a comprehensive screen of all available SNPs on the TNF gene needs to be investigated thoroughly for future gene-disease association studies particularly in the study of Tuberculosis.

CONCLUSION

In conclusion, our pilot study showed that the TNF -238 (rs361525) is not associated with susceptibility to Tuberculosis particularly in the Indonesian population. Although bigger sample size may help in obtaining more reliable results, screening of all SNPs in the TNF gene or other TB susceptibility candidate genes may be a better avenue for future studies.

Limitations of this study

We currently do not supply a known genotype control for the SNP melting analysis, which may affect the accuracy of the genotype calls. Therefore, further assessment of the genotype by sequencing is required to confirm the different genotypes found. Lastly, it is also essential to confirm whether the differences in genotype, affect the gene expression levels of TNF, by monitoring the mRNA levels of this gene initially *in-vitro*. Others have also showed the limitations of single SNP association study (reviewed in (Bayley, Ottenhoff & Verweij 2004)),

therefore we propose the inclusion of all SNPs in the promoter and coding region of TNF gene for further analysis, and assess the association with phenotype by haplotype configurations.

Competing interests

The authors declare that they have no competing interests.

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REFERENCES

Abraham LJ and Kroeger KM 1999. Impact of the-308 TNF promoter polymorphism on the transcriptional regulation of the TNF gene: relevance to disease. J Leukocyte Biol 66 (4): 562-6.

Amirzargar AA, Rezaei N, Jabbari H, Danesh A-A, Khosravi F, Hajabdolbaghi M, Yalda A and Nikbin B 2006. Cytokine single nucleotide polymorphisms in Iranian patients with pulmonary tuberculosis. Eur Cytokine Netw 17 (2): 84-9.

Anoosheh S, Farnia P and Kargar M 2011. Association between TNF-Alpha (-857) Gene Polymorphism and Susceptibility to Tuberculosis. Iranian Red Crescent Medical Journal 13 (4): 243-8.

Ates O, Musellim B, Ongen G and Topal-Sarikaya A 2008. Interleukin-10 and tumor necrosis factor-alpha gene polymorphisms in tuberculosis. J Clin Immunol 28 (3): 232-6.

Baena A, Leung JY, Sullivan AD, Landires I, Vasquez-Luna N, Quinones-Berrocal J, Fraser PA, Uko GP, Delgado JC, Clavijo OP, Thim S, Meshnick SR, Nyirenda T, Yunis EJ and Goldfeld AE 2002. TNF-[alpha] promoter single nucleotide polymorphisms are markers of human ancestry. Genes Immun 3 (8): 482-7.

Bayley JP, Ottenhoff TH and Verweij CL 2004. Is there a future for TNF promoter polymorphisms? Genes Immun 5 (5): 315-29.

Bellamy R 1998. Genetic susceptibility to tuberculosis in human populations. Thorax 53 (7): 588-93.

Brinkman BM, Zuijdeest D, Kaijzel EL, Breedveld FC and Verweij CL 1995. Relevance of the tumor necrosis factor alpha (TNF alpha) -308 promoter polymorphism in TNF alpha gene regulation. J Inflammation 46 (1): 32-41.

Comstock GW 1978. Tuberculosis in twins: a re-analysis of the Prophit survey. The American review of respiratory disease 117 (4): 621-4.

Correa PA, Gomez LM, Cadena J and Anaya JM 2005. Autoimmunity and tuberculosis. Opposite association with TNF polymorphism. J Rheumatol 32 (2): 219-24.

Delgado JC, Baena A, Thim S and Goldfeld AE 2002. Ethnic-specific genetic associations with pulmonary tuberculosis. J Infect Dis 186 (10): 1463-8.

Ding S, Jiang T, He J, Qin B, Lin S and Li L 2012. Tagging single nucleotide polymorphisms in the IRF1 and IRF8 genes and tuberculosis susceptibility. PLoS One 7 (8): e42104.

Elahi MM, Asotra K, Matata BM and Mastana SS 2009. Tumor necrosis factor alpha −308 gene locus promoter polymorphism: An analysis of association with health and disease. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1792 (3): 163-72.

Flesch IE and Kaufmann SH 1990. Activation of tuberculostatic macrophage functions by gamma interferon, interleukin-4, and tumor

necrosis factor. Infect Immun 58 (8): 2675-7.

Flori L, Delahaye NF, Iraqi FA, Hernandez-Valladares M, Fumoux F and Rihet P 2005. TNF as a malaria candidate gene: polymorphism-screening and family-based association analysis of mild malaria attack and parasitemia in Burkina Faso. Genes Immun 6 (6): 472-80.

Flynn JL, Goldstein MM, Chan J, Triebold KJ, Pfeffer K, Lowenstein CJ, Schreiber R, Mak TW and Bloom BR 1995. Tumor necrosis factor-alpha is required in the protective immune response against Mycobacterium tuberculosis in mice. Immunity 2 (6): 561-72.

Goldfeld AE, Doyle C and Maniatis T 1990. Human tumor necrosis factor alpha gene regulation by virus and lipopolysaccharide. Proc Natl Acad Sci U S A 87 (24): 9769-73.

Hatin W, Nur-Shafawati A, Etemad A, Jin W, Qin P, Xu S, Jin L, Tan S-G, Limprasert P, Feisal M, Rizman-Idid M, Zilfalil B and Consortium THP-AS 2014. A genome wide pattern of population structure and admixture in peninsular Malaysia Malays. The HUGO Journal 8 (1): 5.

Henao MI, Montes C, Paris SC and Garcia LF 2006. Cytokine gene polymorphisms in Colombian patients with different clinical presentations of tuberculosis. Tuberculosis (Edinb) 86 (1): 11-9.

Higuchi T, Seki N, Kamizono S, Yamada A, Kimura A, Kato H and Itoh K 1998. Polymorphism of the 5'-flanking region of the human tumor necrosis factor (TNF)-alpha gene in Japanese. Tissue Antigens 51 (6): 605-12.

Höhler T, Kruger A, Gerken G, Schneider PM, Meyer Zum Büschenfelde KH and Rittner C 1998. A tumour necrosis factor-alpha (TNF-α) promoter polymorphism is associated with chronic hepatitis B infection. Clin Exp Immunol 111 (3): 579-82.

Huizinga TW, Westendorp RG, Bollen EL, Keijsers V, Brinkman BM, Langermans JA, Breedveld FC, Verweij CL, van de Gaer L, Dams L, Crusius JB, Garcia-Gonzalez A, van Oosten BW, Polman CH and Pena AS 1997. TNF-alpha promoter polymorphisms, production and susceptibility to multiple sclerosis in different groups of patients. J Neuroimmunol 72 (2): 149-53.

Kaijzel EL, van Krugten MV, Brinkman BM, Huizinga TW, van der Straaten T, Hazes JM, Ziegler-Heitbrock HW, Nedospasov SA, Breedveld FC and Verweij CL 1998. Functional analysis of a human tumor necrosis factor alpha (TNF-alpha) promoter polymorphism related to joint damage in rheumatoid arthritis. Mol Med 4 (11): 724-33.

Kallmann FJ and Reisner D 1943. Twin studies on the significance of genetic factors in tuberculosis. Am Rev Tuberc 47: 549-74.

Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, Siegel JN and Braun MM 2001. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N Engl J Med 345 (15): 1098-104.

Kim HS, Park MH, Song EY, Park H, Kwon SY, Han SK and Shim YS 2005. Association of HLA-DR and HLA-DQ genes with susceptibility to pulmonary tuberculosis in Koreans: preliminary evidence of associations with drug resistance, disease severity, and disease recurrence. Hum Immunol 66 (10): 1074-81.

Kleinnijenhuis J, Oosting M, Joosten LA, Netea MG and Van Crevel R 2011. Innate immune recognition of Mycobacterium tuberculosis. Clin Dev Immunol 2011.

Knight JC, Keating BJ, Rockett KA and Kwiatkowski DP 2003. In vivo characterization of regulatory polymorphisms by allele-specific quantification of RNA polymerase loading. Nat Genet 33 (4): 469-75.

Kobayashi K, Yuliwulandari R, Yanai H, Naka I, Lien LT, Hang NT, Hijikata M, Keicho N and Tokunaga K 2012. Association of TLR polymorphisms with development of tuberculosis in Indonesian females. Tissue Antigens 79 (3): 190-7.

Kroeger KM, Carville KS and Abraham LJ 1997. The− 308 tumor necrosis factor-α promoter polymorphism effects transcription. Mol Immunol 34 (5): 391-9.

Lin PL, Plessner HL, Voitenok NN and Flynn JL 2007. Tumor necrosis factor and tuberculosis. J Investig Dermatol Symp Proc 12 (1): 22-5.

Mekinian A, Tamouza R, Pavy S, Gestermann N, Ittah M, Mariette X and Miceli-Richard C 2011. Functional study of TNF-α promoter polymorphisms: literature review and meta-analysis. Eur Cytokine Netw 22 (2): 88-102.

Mohan VP, Scanga CA, Yu K, Scott HM, Tanaka KE, Tsang E, Tsai MM, Flynn JL and Chan J 2001. Effects of tumor necrosis factor alpha on host immune response in chronic persistent tuberculosis: possible role for limiting pathology. Infect Immun 69 (3): 1847-55.

Morales-Garcia G, Falfan-Valencia R, Garcia-Ramirez RA, Camarena A, Ramirez-Venegas A, Castillejos-Lopez M, Perez-Rodriguez M, Gonzalez-Bonilla C, Grajales-Muniz C, Borja-Aburto V and Mejia-Arangure JM 2012. Pandemic influenza A/H1N1 virus infection and TNF, LTA, IL1B, IL6, IL8, and CCL polymorphisms in Mexican population: a case-control study. BMC Infect Dis 12: 299.

Motulsky AG 1989. Metabolic polymorphisms and the role of infectious diseases in human evolution. Hum Biol: 835-69.

Murray CJ, Styblo K and Rouillon A 1990. Tuberculosis in developing countries: burden, intervention and cost. Bulletin of International Union against Tuberculosis and Lung Disease 65 (1): 6-24.

Oliveira MMd, Silva JCSd, Costa JF, Amim LH, Loredo CCS, Melo H, Queiroz LF, Mello FCQ, Lapa e Silva JR, Kritski AL and Santos AR 2004. Distribuição de Polimorfismos de Base única (SNPs) no gene de TNF-a (-238/-308) entre pacientes com TB e outras pneumopatias: marcadores genéticos de susceptibilidade a ocorrência de TB? Jornal Brasileiro de Pneumologia 30: 371-7.

Pociot F, D'Alfonso S, Compasso S, Scorza R and Richiardi PM 1995. Functional Analysis of a New Polymorphism in the Human TNF α Gene Promoter. Scand J Immunol 42 (4): 501-4.

Selvaraj P, Sriram U, Mathan Kurian S, Reetha AM and Narayanan PR 2001. Tumour necrosis factor alpha (-238 and -308) and beta gene polymorphisms in pulmonary tuberculosis: haplotype analysis with HLA-A, B and DR genes. Tuberculosis (Edinb) 81 (5-6): 335-41.

Skoog T, van't Hooft FM, Kallin B, Nilsson J, Eriksson P and Hamsten A 1999. A common polymorphism in the promoter region of TNF-alpha gene determining the serum TNF-alpha concentration. Atherosclerosis 144: 121.

Stead WW, Senner JW, Reddick WT and Lofgren JP 1990. Racial differences in susceptibility to infection by Mycobacterium tuberculosis. New Engl J Med 322 (7): 422-7.

Uglialoro AM, Turbay D, Pesavento PA, Delgado JC, McKenzie FE, Gribben JG, Hartl D, Yunis EJ and Goldfeld AE 1998. Identification of three new single nucleotide polymorphisms in the human tumor necrosis factor-α gene promoter. Tissue Antigens 52 (4): 359-67.

Vannberg FO, Chapman SJ, Khor CC, Tosh K, Floyd S, Jackson-Sillah D, Crampin A, Sichali L, Bah B, Gustafson P, Aaby P, McAdam KP, Bah-Sow O, Lienhardt C, Sirugo G, Fine P and Hill AV 2008. CD209 genetic polymorphism and

tuberculosis disease. PLoS One 3 (1): e1388.

Vejbaesya S, Chierakul N, Luangtrakool P and Sermduangprateep C 2007. NRAMP1 and TNF-α polymorphisms and susceptibility to tuberculosis in Thais. Respirology 12 (2): 202-6.

WHO 2014. Global Tuberculosis Report 2014. World Health Organization.

Wilson A, Symons J, McDowell T, Di Giovine F and Duff G 1994. Effects of a tumor necrosis factor (TNF-alpha) promoter base transition on transcriptional activity. Br J Rheumatol 33 (suppl 1): 89.

Yea SS, Yang YI, Jang WH, Lee YJ, Bae HS and Paik KH 2001. Association between TNF-alpha promoter polymorphism and Helicobacter pylori cagA subtype infection. J Clin Pathol 54 (9): 703-6.