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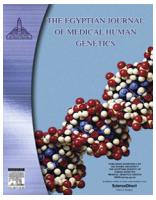

Original article

Association of toll-like receptor 2 polymorphisms with susceptibility to pulmonary tuberculosis in Sudanese

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ABSTRACT

Background: *Mycobacterium tuberculosis* (MT) is a leading cause of death worldwide, and the incidence of the tuberculosis) has been escalating due to the convergence of multidrug-resistant strains and HIV infection epidemics. Genetic and nongenetic factors of both the bacterium and the host have impact on the host response to MT. Toll-like receptors (TLRs) play an important role in the innate immune response to pathogens. It has been proved that viable Mycobacterium bacilli contain distinct ligands that activate cells via TLR2.

Aim: This study was conducted to test the association of TLR2 gene polymorphisms with susceptibility to pulmonary tuberculosis in Sudanese.

Subjects and methods: A case-control study of 207 Sudanese patients with pulmonary tuberculosis and 395 healthy controls was used. Three tag single nucleotide polymorphisms (SNPs) in TLR2 gene and its 3-Kb flanking regions on chromosome 4 were selected. The tag approach was set to r2 > 0.8 and minor allele frequency (MAF) > 0.2. Genotyping was performed by polymerase chain reaction followed by restriction fragment length polymorphism method.

Results: Genotypes of the 3 SNPs (rs1816702, rs3804099, and rs7656411) were estimated and compared. The stepwise regression procedure demonstrated that elimination of rs1816702 and rs3804099 from the model did not have any significant effect (p = .0685, .7300 respectively), while rs7656411 was significantly associated with tuberculosis susceptibility (p = .0372). TLR2 rs7656411 TG and GG genotypes frequencies were higher in pulmonary tuberculosis patients (OR = 1.74, 95% CI = 1.09–2.78, and OR = 2.24, 95% CI = 1.37–3.68 respectively). The haplotype TCG of TLR2 SNPs was also associated with TB susceptibility (p = .0004).

Conclusion: Our study suggests that allele G of rs765641 on TLR2 gene might influence susceptibility to pulmonary tuberculosis in Sudanese.

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1. Introduction

Toll-like receptors (TLRs) are a class of proteins that highly expressed on cells of the immune system and play a crucial role in initiating an effective immune response that protects the host against invading pathogens [1,2]. The TLRs act as key receptors responsible for recognition of specific conserved components of microbes called pathogen-associated molecular patterns (PAMPs), including lipopolysaccharide, lipoproteins, peptidoglycan, CpG DNA, double-stranded RNA, bacterial flagellin [3]. Association of PAMPs with a specific TLR results in receptor dimerization and activation of intracellular signaling cascades, includes the adapter molecule myeloid differentiation primary response protein 88 (MyD88) and (Interleukin-1 receptor) IL-1R-associated kinase (IRAK), MyD88 recruits IRAK to the IL-1R signaling that may use alike molecular cascade for TLRs signaling especially TLR2 and TLR4. IRAK interacts with the adapter molecule TRAF6 (tumour necrosis factor receptor-associated factor 6) that bridges them to the protein kinases TAK1 (transforming growth factor-b-activated kinase) and NIK (NF-κB-inducing kinase). Finally, the protein kinase NIK has been shown to act as a general mediator of TRAF-induced NF-κB activation which results in expression of cytokines, chemokines, and interferons required to activate effector mechanisms both innate and adaptive, leading to the elimination of the

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invading pathogen [4,5]. Recent evidence suggests that TLR2 is important for host defense against *Mycobacterium tuberculosis* (MT) [6].

Genetic changes in TLR sequence have functional repercussions in the immune response and innate immune signaling. The function of TLRs in various human diseases has been investigated by comparison of the incidence of disease among people having different polymorphisms in genes that participate in TLR signaling [7–9]. The polymorphisms within TLR2, TLR4 and TLR5 have been shown to alter protein behavior and thereby increase susceptibility of human populations to diseases such as TB and leprosy, atherosclerosis, cancer and malaria [10,11]. Knockout studies showed that mice deficient in TLR4, TLR2 and TLR9 were found more susceptible to mycobacterial infection [12,13]. TLR2 polymorphisms have shown significant impact on susceptibility or resistance to TB [14].

In this study, we aimed to assess the association of TLR2 polymorphisms and pulmonary tuberculosis in Sudanese.

2. Subjects and methods

2.1. Study population

The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments on humans. The Ethics Committee, Faculty of Medicine, University of Gezira, and the Ethics Committee of The Hong Kong Polytechnic University, Hong Kong, China, approved the study protocol. Written information of the study rational was given to the study participants, then their consent received by signature or thumbprint. This was a case-control study, two hundreds and seven patients with pulmonary TB (146 males and 61 females) were recruited from the Chest Department at Wad Medani Teaching Hospital in Gezira State-Sudan. Diagnosis was done by sputum smear positive of acid-fast bacilli using Ziehl-Neelsen staining technique; all cases were confirmed by two consecutive smear-positive samples. The mean age of cases was 30.0 ± 11.6 years (range: 13–75 years). All the clinical diagnostic procedures and personal data were done at the chest clinic using a structured questionnaire. There were 395 (355 males and 40 females) hospital-based controls. The mean age of the controls was 28.5 ± 6.1 years (range: 18–54 years). The controls completed the questionnaire on their health background about the history of lung diseases including TB, and only individuals free of past or family history of TB infection were allowed to participate in the study. Cases and controls were screened for HIV1/2 by Behring Enzygnost HIV Integral II kit. Immunocompromized individuals with diabetes mellitus, HIV, taking glucocorticoids and/or immunosuppressive therapy were excluded from the study. In order to define a homogeneous phenotype, patients suspected of extra-pulmonary tuberculosis were not considered in the study.

2.2. TLR2 gene and selection of single nucleotide polymorphisms (SNPs)

The human *TLR2* gene is located on chromosome 4q32, (GeneID = 7097, *TLR2* coding sequence, GenBank accession number NM_003264.3). It consists of two 5′ noncoding exons followed by a third coding exon and encodes a putative 784 amino-acid protein. SNPs are the markers of choice in genetic case-control association studies. The genotypes of neighboring SNPs are often highly correlated (in linkage disequilibrium, LD) within a population, which is utilized for selecting specific 'tagSNPs' to serve as proxies for other nearby SNPs in high LD [15]. Although methods based on individual SNPs may lead to significant findings, methods based on haplotypes comprising multiple SNPs on the same inherited chromosome may provide additional power for mapping disease genes [16]. LD TAG SNP Selection tool from National Institutes of Health https://snpinfo.niehs.nih.gov/snpinfo/snptag.html was used. Three tag SNPs (Intronic rs4696483 (also captured by rs1816702), synonymous rs3804099, and 3′UTR rs7656411) in *TLR2* have been selected from the region including 3 Kb upstream and downstream (r^2 threshold > 0.8 and minor allele frequency (MAF) > 0.2).

2.3. 3TLR2 genotyping

The primers were designed using the Oligo software *ver*. 6. Primers were purchased from Invitrogen. Genotyping was carried out by PCR-Restriction fragment length polymorphism (PCR-RFLP), the PCR products were digested by restriction enzymes, which acquired from Fermentas Life Sciences, and visualized on nondenaturing polyacrylamide gel using SYBR green. Primers, digesting enzymes, amplification conditions are shown in Table 2. All genotyping protocols were confirmed by sequencing of 8 samples using the Applied Biosystems 3130 Genetic Analyzer.

2.4. Statistical analysis

A two-step statistic analysis was performed to determine the association of *TLR2* polymorphisms with TB. The genotype frequencies of tag SNPs were compared between TB cases and controls by a *Chi-square* test. Then, the multivariate logistic regression analysis was performed to evaluate the conditional effect of the SNPs (forward and backward procedures) using Stata- GENASS package (*p-values* of less than 0.05 were considered statistically significance). The Bonferroni method was applied to adjust for multiple comparisons of the 3 SNPS; the *p* value was adjusted to 0.017. Haplotype frequency was estimated using the PLINK software [17], conditional haplotype-based testing was executed to specify a particular haplotype to be tested against all others and whether SNPs have an effect that is independent of the other SNPs in the model.

Table 2
Primers used for SNP genotyping for the *TLR2* gene.

SNP	Primer sequence and PCR condition	Amplicon size and Restriction enzyme
rs1816702	F(CTT TTG TGA ATC TGA GTG CTG CC) R(ttttttttttttttttttt GTC TCC ACG ACC GA**C** CTG) 95 °C/5 min] 1x⁻[95 °C/30 s-61/30 s-72 °C/40 s]x3872 °C/5 min] 1x	593/BseLI
rs3804099	F(TTG AGG AAC TTG AGA TTG ATG CTT) R(TGT GAA AGT AAA CAA GGA ACC AGA) 95 °C/5 min] 1x⁻[95 °C/30 s-58/30 s-72 °C/40 s]x3872 °C/ 5 min] 1x	544/TaiI
rs7656411	F(CAT CTT GTT GCT TTT TCC TTC TTC) R(GCC CAA TTA ACC ACA TAC ACC) 95 °C/5 min] 1x⁻[95 °C/30 s-58 /30 s-72 °C/40 s]x3872 °C/5 min] 1x	378/ BspLI

For enzyme recognition, the introduced mismatched base in the primers is bolded and underlined, added poly T tail indicated by small letter t.

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