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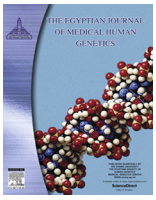

Original article

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.

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.

3. Results

3.1. Profile of cases with tuberculosis and control subjects

The descriptive and clinical features of TB cases in this study are displayed in Table 3.1. The males represented 70.5% (146/207) of cases. The incidence of TB was higher in the age groups ≤20–40 years. Individuals from lower socioeconomic status backgrounds are at greater risk for a variety of poor health outcomes, of all cases included for this study, 179 responded to the question about socioeconomic status, 38 declared they were of middle socioeconomic status, and 140 declared they were of low socio-economic status.

Table 3.1
Characteristics of cases and controls.

	Cases (n = 207)	Controls (n = 395)
Gender (M/F)	146/61	355/40
Age (year, mean ± SD)	30 ± 11.60	28.5 ± 6.10
Onset of symptoms (month, mean ± SD)	12.86 ± 10.99	
Clinical manifestations (%)		
Cough	96.60	
Weight loss	93.70	
Appetite loss	91.80	
Chest pain	89.90	
Fever	84.10	
Malaise	65.70	
Weakness	35.80	
Night sweats	24.60	
Hemoptysis	20.80	
Percentage of cases in the age groups 20-40yrs	82.40	
TB case definition (%)		
New case	87.50	
Relapse	8.70	
Treatment after previous treatment failure	3.80	

M/F: Male/Female, SD: standard deviation.

3.2. Genotype analysis of TLR2 polymorphisms

The genotypes of the 3 SNPs were all in Hardy-Weinberg equilibrium. There were significantly fewer males in the case group than in the control group (70.5% vs. 89.9%; $p < .0001$). To avoid potential confounding due to gender, comparison between cases and controls was performed by stratification into males and females and the results were combined by Mantel-Haenszel procedure. The genotype/allele frequencies of the *TLR2* gene polymorphisms are summarized in Table 3.2. Two SNPs rs1816702 and rs7656411 showed significant differences between cases and controls in genotype frequencies ($p = .0160$ and 0.0050 respectively, at p significance level ≤ 0.017). The reference allele was the less common allele (T) for 2 SNPs rs3804099 and rs7656411, but was the more frequent allele for rs1816702. Comparison of allele frequencies showed significant differences in allele frequency between cases and controls with rs7656411 ($p = .0064$; *OR*, 1.68; *CI*, 1.03–2.75, at p significance level ≤ 0.017). Moreover, a stepwise logistic regression procedure was used to detect which of the significant SNPs contributed to the main effects of the other positive SNPs. By this procedure, elimination of rs1816702 and rs3804099 from the model did not have any significant effect ($p = .0685$, .7300 respectively). That procedure demonstrated that rs7656411 significantly contributed in the development of TB ($p = .0372$).

3.3. Haplotype analysis for TLR2

For the investigated 3 SNPs one haplotype block was generated to get the haplotype frequencies in cases and controls using Plink (7 common haplotypes were obtained at minor haplotype frequency ≥0.01). Odds ratio (OR) was calculated to assess the associations between TB and TLR2 haplotypes (Table 3.3). Haplotypes CTT and TCG differed significantly in frequencies between cases and controls at the nominal cut-off p value of .05 ($p = .0234$, 6.28E−05 respectively), but only TCG showed statistically significant difference after correction for multiple comparisons by

Table 3.2
TLR2 SNPs: comparison of genotype data between cases and controls.

SNP	Genotype	Cases/Controls			Allele	Cases/Controls		
		(207/395)	OR (95% CI)	P value		(414/790)	*OR* (95% CI)	*p* value
rs1816702	CC	81/199	1.00 (reference)	0.016*	C	259/559	1.00 (reference)	0.0267
	CT	97/161	1.48 (1.03–2.13)		T	155/231	1.35 (0.93–1.96)	
	TT	29/35	2.04 (1.16–3.57)					
rs3804099	TT	32/82	1.00 (reference)	0.102	T	150/339	1.00 (reference)	0.0402
	TC	86/175	1.26 (0.78–2.04)		C	264/451	1.12 (0.66–1.89)	
	CC	89/138	1.65 (1.02–2.69)					
rs7656411	TT	32/103	1.00 (reference)	0.005*	T	163/389	1.00 (reference)	0.0064*
	TG	99/183	1.74 (1.09–2.78)		G	251/401	1.68 (1.03–2.75)	
	GG	76/109	2.24 (1.37–3.68)					

* Significant (the significance level adjusted for the 3 SNPs by Bonferroni procedure to 0.017).

Table 3.3
Haplotype frequencies of the *TLR2* gene among cases and controls.

Haplotype	Estimated freq in cases (n = 414)	Estimated freq in controls (n = 790)	OR	$\chi 2$ test *p* value	Empirical *p* value
TTT	0.038	0.033	1.17 Ref.	0.6292	0.9987
CTT	0.272	0.336	0.65	0.0234*	0.1344
TCT	0.035	0.06	0.37	0.0595	0.2977
CCT	0.049	0.064	0.71	0.2912	0.8734
CTG	0.051	0.058	0.71	0.5926	0.9971
TCG	0.30	0.198	1.24	6.28E−05*	0.0004*
CCG	0.254	0.25	0.78	0.8690	1.000

Abbreviations: OR, odds ratio; Empirical p value, haplotypes of cases and controls were permuted 100,000 times (the p value is estimated by n = 100,000).
* Indicates significant at 0.05

permutation (p = .0004). The haplotype CTT was present at a lower frequency in cases than in controls (0.27 vs. 0.34) and consequently conferred protection while the haplotype TCG was present at higher frequency in cases than in controls (0.30 vs. 0.198) and consequently conferred susceptibility to TB.

4. Discussion

According to our data, we found that 81.6% of cases were aged 40 years or younger, male gender contributed about 70.5% to the total numbers, and 78% were of low socioeconomic status. Hence, the younger age group, male gender, and low socioeconomic status are high risk factors for TB. The association of TB with male sex is in agreement with what has been reported in most countries in Africa (Guinee, Guinea Bissau, and The Gambia), where rates in males higher than those of females [18,19]. In Africa, a genome-wide linkage study searching for regions of the human genome containing TB susceptibility genes suggested a linkage between regions of the *chromosome X* and TB, with a lod score of 1.77, which could contribute to the excess of TB in males in many populations [20]. TLR8 gene polymorphisms on *chromosome X* showed association with TB susceptibility in males [21]. In cases the mean age was 3 0.0 ± 11.6 years. It has been demonstrated that older age is associated with an increased risk of TB infection progressing to disease [22]. There seems to be a distinct pattern of risk of developing TB over a life time which has the shape of an inverted U. With the exception of the infant years, the risk of TB is lowest in later childhood but rises rapidly to peak at about age 25–35 years. Thereafter rates decline progressively [23]. Our incidence rates, increasing with age, would correspond the data from sub-Saharan Africa community of Guinea-Bissau [24]. The majority of our cases were of low socioeconomic status. The TB burden follows a strong socioeconomic gradient between countries, within countries, and within communities, and the poorest have the highest risk [25]. Both individual and community-level may play an important role in determining an individual's risk of becoming ill with TB [26]. It seems that male sex, middle age, and low socio-economic status are predisposing factors for TB.

The genetic susceptibility to TB has been addressed by investigating the relationship between *TLR2* polymorphisms in Sudanese population. We examined 3 markers (*tag SNPs*) in the *TLR2* gene for association with TB. We observed statistically significant associations in *TLR2* due to the difference in the genotype frequencies at rs7656411 between cases and controls. The Genetic Power Calculator [27] showed that 203 cases and 385 controls (a control:case ratio of 1.9) would be sufficient to have 80% power to observe an association for rs7656411. Allele frequency data for the 3 SNPs in dbSNP database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=snp) were retrieved for comparison. As expected, the allele frequencies of rs7656411 in our population were similar to those from the Yoruba people in Ibadan, Nigeria (G = 0.608 and T = 0.392). The Han Chinese population has similar allele frequencies to those from Africa, where G is the major allele (0.589) and T is the minor allele (0.411). Conversely, in European population T is the more frequent allele (0.797), and thus the frequency of the risk allele at rs7656411 seemed to be high among African and Chinese populations. This analysis infers that the polymorphisms of rs7656411 in downstream region (3′UTR) set challenges for establishing the mechanism by which this locus influences the disease susceptibility.

Haplotype patterns analysis is widely used in association studies [28]; for the 3 SNPs [rs1816702, rs3804099 and rs7656411] inspected 2 haplotypes were associated with susceptibility to pulmonary TB: CTT and TCG. Assigning haplotypes according to their frequencies and odds ratio the CTT was a protective haplotype while the TCG was a high-risk haplotype. With reference to CTT and TCT, the 2 most different haplotypes in controls, rs1816702 and rs3804099 were unlikely to exert the possible protective effect. This is consistent with the single-marker analysis, the protective haplotype carried the T allele of rs7656411, and the G allele of rs7656411 was present in the risk haplotype. Interestingly, we found different genetically skewed haplotype, the TCG risk haplotype carried the common allele G of rs7656411, while the protective haplotype CTT carried the minor allele T. It is suggested that *TLR2* rs7656411 G allele and further associated haplotype including this SNP were significantly associated with TB and possibly representing a susceptibility locus. To predict the functional importance of rs7656411, the bioinformatics tool (SNP functional prediction; https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html) was used but did not recognize any putative effect on *TLR2* expression for rs7656411. It was shown that rs7656411 in the 3′UTR is located within the neighboring gene RNF175, a member of RING finger proteins which are suggested to act as E3 ubiquitin ligases that expedite selective autophagy of MT [29]. Mutations/ polymorphisms affecting secondary structure of 3′UTR of mRNA can cause translational de-regulation and disease. Likewise, microRNAs post-transcriptionally regulate gene expression by binding to their target mRNAs (usually within the 3′UTRs) [30]. In line with our findings, it was reported that *TLR2* protein expression was modulated due to miR-105 targeting the 3′UTR [31].

A study examined the association of *TLR2* polymorphisms and the development of active TB in Caucasians, African-Americans, and West Africans (Guinea-Bissau) [32]. Ten SNPs were genotyped in the 3 populations. Our 3 selected polymorphisms rs1816702, rs3804099 and rs7656411 of *TLR2* were genotyped in these populations, 2 SNPs rs3804099 and rs7656411showed statistically significant associations in samples from Guinea-Bissau. Several reports showed the *TLR2* polymorphisms have been implicated in susceptibility to TB. The frequencies of a human *TLR2* Arg677Trp (rs5743706) and Arg753Gln (rs4986790) polymorphisms in TB patients were significantly different from healthy controls in populations from Tunis and Turkey respectively [33,34]. Nevertheless, these 2 polymorphisms were not found responsible for the increased prevalence of TB in the Indian population [35]. Thuong et al. found strong association of *TLR2* rs3804099 with the development of TB meningitis and miliary TB [7]. In Korea, the development of TB disease was associated with shorter GT repeats in intron II of the *TLR2* gene; luciferase activities for different GT repeats demonstrated a correlation of lower TLR2 expression and shorter repeats [36]. It was also shown that the insertion/deletion polymorphisms in *TLR2* (−196 to −174) in the 5′-untranslated region influence the risk for pulmonary TB in Caucasians [32]. Though all the rs7656411 is not in LD with the above studied polymorphisms, more validating studies are needed to divulge the potential role of *TLR2* in TB susceptibility.

5. Conclusions

Our data suggest that *TLR2* rs7656411 G allele and further associated haplotype were significantly associated with TB. Additionally, the rs7656411 is located within the neighboring gene RNF175. The association with TB may reflect the functional role of rs7656411, or this tag SNP probably in linkage disequilibrium with the causal variant(s) in this region.

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Author contributions

H. Zaki and N. Gasmelseed designed the study and wrote the protocol. S. P. Yip facilitated the practical part and supervised all the steps of this work. B. Abdalla revised the manuscript. All authors approved the final manuscript.

Conflicts of interest statement

The authors confirm that there are no known conflicts of interest.

References

[1] Akira S. Mammalian toll-like receptors. Curr Opin Immunol 2003;15(1):5–11.
[2] Saleh MA, Ramadan MM, Arram EO. Toll-like receptor-2 Arg753Gln and Arg677Trp polymorphisms and susceptibility to pulmonary and peritoneal tuberculosis. APMIS 2017;125(6):558–64.
[3] Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol 2003;21:335–76.
[4] Medzhitov R. Toll-like receptors and innate immunity. Nat Rev Immunol 2001;1(2):135–45.
[5] Muzio M, Polentarutti N, Bosisio D, Manoj Kumar PP, Mantovani A. Toll-like receptor family and signalling pathway. Biochem Soc Trans 2000;28(5):563–6.
[6] Konowich J, Gopalakrishnan A, Dietzold J, Verma S, Bhatt K, Rafi W, et al. Divergent functions of TLR2 on hematopoietic and nonhematopoietic cells during chronic Mycobacterium tuberculosis infection. J Immunol 2017;198(2):741–8.
[7] Thuong NT, Hawn TR, Thwaites GE, Chau TT, Lan NT, Quy HT, et al. A polymorphism in human TLR2 is associated with increased susceptibility to tuberculous meningitis. Genes Immun 2007;8(5):422–8.
[8] Hernesniemi JA, Raitakari OT, Kahonen M, Juonala M, Hutri-Kahonen N, Marniemi J, et al. Toll-like receptor 4 gene (Asp299Gly) polymorphism associates with carotid artery elasticity. The cardiovascular risk in young Finns study. Atherosclerosis 2008;198(1):152–9.
[9] Mockenhaupt FP, Cramer JP, Hamann L, Stegemann MS, Eckert J, Oh NR, et al. Toll-like receptor (TLR) polymorphisms in African children: common TLR-4 variants predispose to severe malaria. Proc Natl Acad Sci USA 2006;103(1):177–82.
[10] Misch EA, Hawn TR. Toll-like receptor polymorphisms and susceptibility to human disease. Clin Sci (Lond) 2008;114(5):347–60.
[11] Khalilullah SA, Harapan H, Hasan NA, Winardi W, Ichsan I, Mulyadi M. Host genome polymorphisms and tuberculosis infection: what we have to say? Egypt J Chest Dis Tuberc 2014;63(1):173–85.
[12] Abel B, Thieblemont N, Quesniaux VJ, Brown N, Mpagi J, Miyake K, et al. Toll-like receptor 4 expression is required to control chronic Mycobacterium tuberculosis infection in mice. J Immunol 2002;169(6):3155–62.
[13] Bafica A, Scanga CA, Feng CG, Leifer C, Cheever A, Sher A. TLR9 regulates Th1 responses and cooperates with TLR2 in mediating optimal resistance to Mycobacterium tuberculosis. J Exp Med 2005;202(12):1715–24.
[14] Bhaladhare A, Sharma D, Kumar A, Sonwane A, Chauhan A, Singh R, et al. Single nucleotide polymorphisms in toll-like receptor genes and case-control association studies with bovine tuberculosis. Vet World 2016;9(5):458–64.
[15] Pettersson FH, Anderson CA, Clarke GM, Barrett JC, Cardon LR, Morris AP, et al. Marker selection for genetic case-control association studies. Nat Protoc 2009;4(5):743–52.
[16] Liu N, Zhang K, Zhao H. Haplotype-association analysis. Adv Genet 2008;60:335–405.
[17] Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81(3):559–75.
[18] Holmes CB, Hausler H, Nunn P. A review of sex differences in the epidemiology of tuberculosis. Int J Tuberc Lung Dis 1998;2(2):96–104.
[19] Lienhardt C, Fielding K, Sillah JS, Bah B, Gustafson P, Warndorff D, et al. Investigation of the risk factors for tuberculosis: a case-control study in three countries in West Africa. Int J Epidemiol 2005;34(4):914–23.
[20] Bellamy R, Beyers N, McAdam KP, Ruwende C, Gie R, Samaai P, et al. Genetic susceptibility to tuberculosis in Africans: a genome-wide scan. Proc Natl Acad Sci USA 2000;97(14):8005–9.
[21] Davila S, Hibberd ML, Hari Dass R, Wong HE, Sahiratmadja E, Bonnard C, et al. Genetic association and expression studies indicate a role of toll-like receptor 8 in pulmonary tuberculosis. PLoS Genet 2008;4(10):e1000218.
[22] Tocque K, Convrey RP, Bellis MA, Beeching NJ, Davies PD. Elevated mortality following diagnosis with a treatable disease: tuberculosis. Int J Tuberc Lung Dis 2005;9(7):797–802.
[23] Davies PD. Risk factors for tuberculosis. Monaldi Arch Chest Dis 2005;63(1):37–46.
[24] Gustafson P, Gomes VF, Vieira CS, Rabna P, Seng R, Johansson P, et al. Tuberculosis in Bissau: incidence and risk factors in an urban community in sub-Saharan Africa. Int J Epidemiol 2004;33(1):163–72.
[25] Lonnroth K, Jaramillo E, Williams BG, Dye C, Raviglione M. Drivers of tuberculosis epidemics: the role of risk factors and social determinants. Soc Sci Med 2009;68(12):2240–6.
[26] Harling G, Ehrlich R, Myer L. The social epidemiology of tuberculosis in South Africa: a multilevel analysis. Soc Sci Med 2008;66(2):492–505.
[27] Purcell S, Cherny SS, Sham PC. Genetic power calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 2003;19(1):149–50.
[28] Beaty TH, Fallin MD, Hetmanski JB, McIntosh I, Chong SS, Ingersoll R, et al. Haplotype diversity in 11 candidate genes across four populations. Genetics 2005;171(1):259–67.
[29] Franco LH, Nair VR, Scharn CR, Xavier RJ, Torrealba JR, Shiloh MU, et al. The ubiquitin ligase Smurf1 functions in selective autophagy of Mycobacterium tuberculosis and anti-tuberculous host defense. Cell Host Microbe 2017;22(3):421–3.
[30] Valencia-Sanchez MA, Liu J, Hannon GJ, Parker R. Control of translation and mRNA degradation by miRNAs and siRNAs. Genes Dev 2006;20(5):515–24.
[31] Benakanakere MR, Li Q, Eskan MA, Singh AV, Zhao J, Galicia JC, et al. Modulation of TLR2 protein expression by miR-105 in human oral keratinocytes. J Biol Chem 2009;284(34):23107–15.
[32] Velez DR, Wejse C, Stryjewski ME, Abbate E, Hulme WF, Myers JL, et al. Variants in toll-like receptors 2 and 9 influence susceptibility to pulmonary tuberculosis in Caucasians, African-Americans, and West Africans. Hum Genet 2010;127(1):65–73.
[33] Ben-Ali M, Barbouche MR, Bousnina S, Chabbou A, Dellagi K. Toll-like receptor 2 Arg677Trp polymorphism is associated with susceptibility to tuberculosis in Tunisian patients. Clin Diagn Lab Immunol 2004;11(3):625–6.
[34] Ogus AC, Yoldas B, Ozdemir T, Uguz A, Olcen S, Keser I, et al. The Arg753GLn polymorphism of the human toll-like receptor 2 gene in tuberculosis disease. Eur Respir J 2004;23(2):219–23.
[35] Biswas D, Gupta SK, Sindhwani G, Patras A. TLR2 polymorphisms, Arg753Gln and Arg677Trp, are not associated with increased burden of tuberculosis in Indian patients. BMC Res Notes 2009;2:162.
[36] Yim JJ, Lee HW, Lee HS, Kim YW, Han SK, Shim YS, et al. The association between microsatellite polymorphisms in intron II of the human Toll-like receptor 2 gene and tuberculosis among Koreans. Genes Immun 2006;7(2):150–5.