Association of Toll –like Receptors 1, 2, 4, 6 ,8, 9 and 10 Genes Polymorphisms and Susceptibility to Tuberculosis in Sudanese Patients

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Research Article

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Abstract

Background: Genetic factors are important contributors to the development of a wide range of complex disease. Polymorphisms in genes encoding for toll like receptors (TLRs) usually influence the efficiency of the immune response to infection and are associated with disease susceptibility and progression. Therefore, we aims to describe the first association between TLR1, TLR2, TLR4 TLR6 , TLR8 , TLR9 ,and TLR10 genes polymorphisms and susceptibility to tuberculosis in Sudanese patients.

Methodology: Here we performed a case study which included 160 tuberculosis patients and 220 healthy matched controls from Sudan. In the study population, we evaluated the possible association between 86 markers in TLR1, TLR2, TLR4 TLR6 , TLR8 , TLR9 ,and TLR10 genes polymorphisms and susceptibility to tuberculosis disease in Sudanese population using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) .

Result: From our results It appeared that in the tuberculosis population the TLR1 rs5743557, TLR1 rs4833095, TLR1 rs5743596, TLR2 rs5743704, TLR2 rs5743708, TLR2 rs3804099 , TLR4 rs4986790 ,TLR4 rs4986791, TLR6 rs5743810 , TLR8 rs3764879 , TLR8 rs3764880, TLR9 rs352165, TLR9 rs352167, TLR9 rs187084 and TLR10 rs4129009 were significantly more often encountered ($p < 0.0001$) than in the control population and were associated with tuberculosis in the Sudanese population. For the other polymorphisms tested, no association with tuberculosis was found in the population tested.

Conclusion: This indicates that the genotypes obtained for TLR1 rs5743557, TLR1 rs4833095, TLR1 rs5743596, TLR2 rs5743704, TLR2 rs5743708, TLR2 rs3804099 , TLR4 rs4986790 ,TLR4 rs4986791, TLR6 rs5743810 , TLR8 rs3764879 , TLR8 rs3764880, TLR9 rs352165, TLR9 rs352167, TLR9 rs187084 and TLR10 rs4129009 allele have a significant role in the genetic susceptibility to development tuberculosis in Sudanese population.

Background

Tuberculosis (TB) has been declared a major public health problem worldwide, due to the emergence of almost untreatable strains of *Mycobacterium tuberculosis* (*M. tuberculosis*). In addition, to that the only existing vaccine against TB, the BCG vaccine, fails to protect against pulmonary TB, the most important form of disease manifestation [1]. It kills approximately 10 million people and nine million of new TB cases are reported worldwide annually [1, 2].

In the last two decades, three major coronavirus epidemics have been reported worldwide [3]. The burden of COVID-19, and TB is one of the major and persistent global health challenges of the twenty-first century [4, 5].

The intersecting coronavirus and TB epidemics in countries with a high burden of TB infections pose several public health challenges. In fact, TB is the leading immune suppressing infection and the most common cause of death among HIV-infected patients [6]. WHO estimated that 1.1 million people worldwide live with TB and HIV, 80% of whom live in sub-Saharan Africa [7, 8]. and causing a high mortality rate among people living with HIV/AIDS over the last ten years [9] Populations infected with HIV and TB, those with undiagnosed pulmonary TB (PTB), drug resistant tuberculosis or complex presentations such as disseminated types and those who have only started PTB treatment may be at elevated risk for severe responses if they are infected with COVID-19 [10]. In the future, lung lesions associated with COVID-19 may increase the risk of PTB, which induces a true fierce circle of HIVTB-COVID-19 co-infection.

Genetic factors are important contributors to the development of a wide range of complex disease [11, 12]. A person who is susceptible to a particular infectious disease, such as TB, the risk of developing the disease is higher than one who has not inherited the genetic risk factor [11, 12]. Several lines of evidence had suggested the importance of host genetic factors in TB susceptibility including the most striking example of a population acquiring resistance to TB occurred in the population of Qu' Appelle Indians [13], twin studies [14], genome-wide linkage studies [15–19], and recently published genome-wide association studies (GWAS) [20, 21].

These evidences on the influence of host genetic factors in TB susceptibility led to the development of strategies to identify candidate genes or susceptibility loci in the human genome. Identification of polymorphisms in genes has enabled linkage and association studies to be used in explaining individual variation in susceptibility to and severity of TB in humans.

In this context, toll like receptors (TLRs) are a family of proteins that are expressed either on the extra cellular cell surface (TLR1, 2, 4, 5, 6) or in the cytosol or on endosomal membranes (TLR3, 7, 8, 9) of macrophages and dendritic cells. TLRs are essential for recognition of a broad repertoire of pathogen-associated molecular patterns (PAMPs) on macrophages and dendritic cells and play an important role in the innate responses against *M. tuberculosis* [22–26]. Genetic variations of TLR1, TLR2, TLR4, TLR6, TLR8, TLR9 and TLR10 have been associated with the susceptibility to TB in different ethnic groups [27–33]. In contrast, other studies have failed to demonstrate significant associations of TLRs polymorphisms with TB [34–37].

Hence, the identification of mutations in these receptors could be used as a marker to screen the individuals who are at risk. Therefore the aim of the present study is to determine the possible association between 86 single-nucleotide polymorphisms (SNPs) of TLR1, TLR2, TLR4 TLR6, TLR8, TLR9 ,and TLR10 with pulmonary tuberculosis in a sub-Saharan Sudanese tuberculosis patients .

Materials And Methods

Study population

A prospective, cross sectional, case–control study was carried out during the period between 2019 and 2020 at Abu-Angah Hospital , Khartoum, Sudan. 160 patients with active pulmonary TB and 220 healthy controls were included. EDTA blood samples were taken from all patients and healthy controls. All tuberculosis patients had microbiological (by culture and/or smear) or radiological evidence of *M. tuberculosis* disease (table 1).

The healthy controls had no evidence of tuberculosis disease by clinical examination, and were matched on age, gender and BCG status (table 1).

The collected blood samples were tested for other infectious diseases and that included hepatitis B (HBsAg, InTec products, INC, China), hepatitis C (Rapid Anti-HCV Test, InTec products, INC, China), syphilis (RAPIDAN TESTER, product code: RTTP01, Turkey), and HIV (HIV1, 2 Cassete test, Clinotech Diagnostics & Pharmaceuticals, Canada). Blood samples were stored at -20° C until use. Nasopharyngeal swab for was used to define Corona Vlrus Disease-2019 (COVID-19)

Ethics statement

The present study was approved by the Ethics Committee of University of Khartoum, Khartoum, Sudan (5/2018). Written informed consents were obtained from all participants in the study.

DNA isolation

Genomic DNA was isolated from blood samples with the large volume kit for the MagNA Pure system (Roche, Almere, The Netherlands) according to the manufacturer's descriptions. The isolated DNA was stored at -20°C.

Genotyping

Genomic variants of TLR1(rs5743604, rs5743611, rs5743618, rs76600635, rs5743551, rs5743557, rs5743565, rs5743566, rs5743580, rs5743594 rs4833095, rs5743595, rs5743596); TLR2 (rs1816702, rs5743704 rs5743708, rs7656411, rs11938228, rs893629, rs1898830,rs121917864, rs4696480, rs3804099, rs5743699 rs3804100); TLR4 (rs7869402, rs1927907, rs1927911, rs1927914, rs6478317, rs55912718, rs5030719, rs10759931, rs10759933, rs2770150, rs1554973, rs11536878, rs11536879, rs7873784, rs11536889, rs4986790, rs4986791, rs11536897, rs11536898); TLR6 (rs3796508,rs5743810, rs5743831, rs1039559, rs6531670, rs5743788, rs5743794); TLR8 (rs4830805, rs4830808, rs3747414, rs3761624, rs1548731, rs2109134, rs3788935, rs1013150, rs5744068, rs3764879, rs5744080, rs3764880, rs17256081, rs5741883, rs2407992, rs178998); TLR9 (rs5743836, rs164637, rs352139, rs352140, rs352143, rs352162, rs352165, rs352167, rs187084); and TLR10 (rs4129009, rs7694115, rs10856839, rs11466645, rs4274855, rs11096955, rs11096956, rs11096957, rs7698870, and rs10776483) genes were detected by PCR followed by restriction enzyme fragment analysis (PCR-RFLP). All PCR primers are stated in Table 2. Each of the PCRs consisted

of a pre-denaturation step of 4 minutes at 94°C and 40 cycles each of 30 seconds denaturation at 94°C, 30 seconds annealing at 55°C and 30 seconds elongation at 72°C. This was followed by a post-elongation step of 7 minutes at 72°C. Restriction endonucleases were selected using the NEBcutter software (<http://nc2.neb.com/NEBcutter2/>). Restriction endonucleases were obtained from Fermentas (st. Leon-rot, Germany), and Roche (Penzberg, Germany) and were used as described by the manufacturer. Restriction fragments were visualized by electrophoresis on 2% agarose gels (Hispanagar, Sphaero Q, Leiden, The Netherlands).

Statistical analysis

The mean age of the patient population and the control population were compared by the unpaired t-test. Gender, occupation and BCG-vaccination status between the patient and control population were compared with the Fisher exact test. Verification of Hardy-Weinberg equilibrium (HWE) was performed with Pearson's χ^2 test. The effect of the TLR1(rs5743604, rs5743611, rs5743618, rs76600635, rs5743551, rs5743557, rs5743565, rs5743566, rs5743580, rs5743594 rs4833095, rs5743595, rs5743596); TLR2 (rs1816702, rs5743704 rs5743708, rs7656411, rs11938228, rs893629, rs1898830,rs121917864, rs4696480, rs3804099, rs5743699 rs3804100); TLR4 (rs7869402, rs1927907, rs1927911, rs1927914, rs6478317, rs55912718, rs5030719, rs10759931, rs10759933, rs2770150, rs1554973, rs11536878, rs11536879, rs7873784, rs11536889, rs4986790, rs4986791, rs11536897, rs11536898);TLR6 (rs3796508,rs5743810, rs5743831, rs1039559, rs6531670, rs5743788, rs5743794); TLR8 (rs4830805, rs4830808, rs3747414, rs3761624, rs1548731, rs2109134, rs3788935, rs1013150, rs5744068, rs3764879, rs5744080, rs3764880, rs17256081, rs5741883, rs2407992, rs178998); TLR9 (rs5743836, rs164637, rs352139, rs352140, rs352143, rs352162, rs352165, rs352167, rs187084); and TLR10 (rs4129009, rs7694115, rs10856839, rs11466645, rs4274855, rs11096955, rs11096956, rs11096957, rs7698870, and rs10776483) polymorphisms on susceptibility to tuberculosis were assessed with the Fisher exact test. P-value of <0.05 was deemed statistically significant All statistical analyses were performed using SPSS for Windows v11.0 statistical analysis software.

Results

Characteristics of tuberculosis patients and healthy control subjects

One hundred and sixty Sudanese tuberculosis patients were included into the study. The diagnosis of tuberculosis was based on the presence of *MTB* in a positive Ziehl-Nielsen (ZN) smear of a sputum specimen and/or by positive culture with tuberculosis and radiological evidence (chest X-ray) (table 1).The control population comprised 220 healthy unrelated people from the same endemic area in Sudan, they were matched on gender and BCG-status (table 1) and showed no signs of any lung disease. Unfortunately the occupation of the control population differed from that of the patient population.

Distribution of TLR1, TLR2, TLR4 TLR6 , TLR8 , TLR9 ,and TLR10 genes polymorphisms:

To detect the possible deficiencies in TLR1(rs5743604, rs5743611, rs5743618, rs76600635, rs5743551, rs5743557, rs5743565, rs5743566, rs5743580, rs5743594 rs4833095, rs5743595, rs5743596); TLR2 (rs1816702, rs5743704 rs5743708, rs7656411, rs11938228, rs893629, rs1898830,rs121917864, rs4696480, rs3804099, rs5743699 rs3804100); TLR4 (rs7869402, rs1927907, rs1927911, rs1927914, rs6478317, rs55912718, rs5030719, rs10759931, rs10759933, rs2770150, rs1554973, rs11536878, rs11536879, rs7873784, rs11536889, rs4986790, rs4986791, rs11536897, rs11536898); TLR6 (rs3796508,rs5743810, rs5743831, rs1039559, rs6531670, rs5743788, rs5743794) TLR8 (rs4830805, rs4830808, rs3747414, rs3761624, rs1548731, rs2109134, rs3788935, rs1013150, rs5744068, rs3764879, rs5744080, rs3764880, rs17256081, rs5741883, rs2407992, rs178998); TLR9 (rs5743836, rs164637, rs352139, rs352140, rs352143, rs352162, rs352165, rs352167, rs187084); and TLR10 (rs4129009, rs7694115, rs10856839, rs11466645, rs4274855, rs11096955, rs11096956, rs11096957, rs7698870, and rs10776483) production among tuberculosis patients, genotype (table 3) and allele frequencies (table 4) in the promoters of the genes encoding for TLR1, TLR2, TLR4, and TLR6 were determined. To determine if the SNPs reached Hardy-Weinberg equilibrium (HWE), the Pearson's χ^2 test was performed. It appeared that in the patients and control population, all genotype distributions were in Hardy-Weinberg disequilibrium.

To determine if there was an association between any of the studied SNPs and tuberculosis, the allele frequencies between the control population and the patient population were compared with the Fisher Exact test. It appeared that in the tuberculosis population the TLR1 rs5743557, TLR1 rs4833095, TLR1 rs5743596, TLR2 rs5743704, TLR2 rs5743708, TLR2 rs3804099, TLR4 rs4986790, TLR4 rs4986791, TLR6 rs5743810, TLR8 rs3764879, TLR8 rs3764880, TLR9 rs352165, TLR9 rs352167, TLR9 rs187084 and TLR10 rs4129009 were significantly more often encountered ($p < 0.0001$) (Table 4) than in the control population and were associated with tuberculosis in the Sudanese population (Table 4). For the other polymorphisms tested, no association with tuberculosis was found in the population tested (Table 4).

Discussion

Association between TLR1, TLR2, TLR4 TLR6, TLR8, TLR9, and TLR10 genes polymorphisms and tuberculosis:

Tuberculosis is a complex disease in which environmental, immunological and genetic factors are contributed. It has been estimated that 10% of the infected population with *M. tuberculosis* may develop TB disease sometime in their life, suggesting that the majority of those infected are endowed with a protective immune response. Previous association studies have indicated a potential involvement of genetic variation within innate immune response genes as risk factors for TB. In particular, TLR1 [38], TLR2 [39–44] TLR4 [45], with TLR8 [46] and TLR9 [30] genetic variants have been associated with TB, suggesting that TLR-mediated responses may be important for protection to *M. tuberculosis* infection in humans.

TLRs are a family of PRRs consisting of 12 members in human and other mammals. TLRs play a crucial role in the recognition of *M. tuberculosis*, this immune activation occurs only in the presence of functional TLRs. Variants of TLRs may influence their expression, function and alters the recognition or signaling mechanism, which leads to the disease susceptibility [47, 48]. The polymorphisms of TLRs have been hypothesized to affect the tuberculosis susceptibility. However, the direct evidence remains controversial.

Therefore, in the present study, we genotyped 86 SNPs including TLR1, TLR2, TLR4 TLR6, TLR8, TLR9, and TLR10 in the Sudanese tuberculosis patients to determine whether they are associated with susceptibility to TB in Sudanese tuberculosis patients.

Our results revealed that TLR1 rs5743557, TLR1 rs4833095, and TLR1 rs5743596 allele were more frequently found in the patients population compared to the healthy controls population. The association of the TLR1 rs5743557, TLR1 rs4833095, and TLR1 rs5743596 allele with tuberculosis were also found in other populations originating from Caucasian, Indian population and South Asian for TLR1 rs4833095 [32, 49] and, East Asian Population for TLR1 rs5743557 [32], but not in populations originating from in North China [32] and African American subpopulation [38]. Further, *in vitro* experiments showed that the TLR1 rs4833095 is in strong linkage disequilibrium (LD) with TLR1 rs5743618 and determining how these two SNPs contribute to TB susceptibility has proven difficult. TLR1 rs5743618 -GT genotype was related to reduction in surface expression of TLR1 in monocytes and granulocytes. In addition, after stimulated by inactivated H37Rv, samples from children with the rs5743618-GT genotype showed a decreased production of TNF- α and CXCL10, invariable production of IL-6 and IL-8 and increased production of IL-10 [50]. In Spanish population, rs5743618 G allele and GG genotype influenced the susceptibility to PTB [33]; in Han Chinese population, Ma *et al.* reported that rs5743618 was not associated with adult TB [38]. Zhang *et al.* [32] conducted a meta-analysis on TLR1 rs5743618 and also found no association with TB susceptibility, which corresponds with our results [32]. Salie *et al.* performed an association study of 23 polymorphisms in five TLR genes (TLR1, TLR2, TLR4, TLR8, and TLR9) in TB cases and healthy controls in a South African population. The study found that TLR1 rs5743618, TLR8 rs3764879, and TLR8 rs3764880 polymorphisms were associated with TB susceptibility in both sexes [51].

Our results also revealed that TLR2 rs5743708, TLR2 rs3804099 were more frequently found in the patient population compared to the healthy control population. The association of the TLR2 rs5743708, with tuberculosis were also found in other populations originating from Asian and Hispanic populations [39, 52, 53] but not European subgroup [51]. Zhang *et al.* [32] showed a significant association with TB susceptibility for the rs5743708 A allele and AA genotype across different ethnic groups in Asians and Europeans, but decreased risk in the Hispanic population [32].

In contrast, the TLR2 rs5743708 and TLR2 rs5743704 were not associated with TB meningitis in an Indian population.[36, 54] TLR2 rs3804099 was associated with susceptibility to TB meningitis rather than with susceptibility to pulmonary TB in a case-control study of a Chinese cohort.[55] Another study investigated possible associations of 16 polymorphisms of six TLR genes and TIRAP with TB susceptibility in a Chinese population. It found that TLR2 rs5743708, polymorphism was associated with pulmonary TB [52].

Several studies have demonstrated critical role of TLR4 in *M. tuberculosis* recognition and verified necessity of these TLR for development of a protective response against *M. tuberculosis* infection [40]. Variants in TLR4, rs4986790 and rs4986791, were investigated for their association with susceptibility or resistance to pulmonary tuberculosis [27, 28, 30, 34, 45].

The results of our study are consistent with the reports of Najmi *et al.* in India [28] Ferwerda *et al.* in Tanzania [45] and Pulido *et al.* in Spain [59] but not similar to that reported previously in other Sudanese [31] and South India [56] Asian populations [34] European Caucasians [34], North and South American [34, 38], African populations [34, 38], Gambian TB population [57], a south-eastern Chinese population [58], Mexico [35], and USA[30].

It is known that for TLR6 rs5743810 SNP have a protective effect against TB development. The T allele was found by Shey *et al.* [60] to reduce NF- κ B signalling which led to an altered level of IL-6 production, while Randhawa *et al.* [61] showed that it leads to increased IFN- γ production and thus protection against *M. tuberculosis*. These functional studies correlate with the results found in this meta-analysis as well as that of Zhang *et al.* [32] where the T allele and TT genotype was also associated with resistance to TB disease.

The TLR8 polymorphism plays a significant part in the immune response .in regulating the induction of interferon (IFN) and inflammatory cytokines [46, 51]. Our results revealed that TLR8 rs3764879, and rs3764880 allele were more frequently found in the patients population compared to the healthy controls population. The association of the TLR8 rs3764879 and rs3764880 alleles with tuberculosis were also found in other populations originating from Russian and Indonesian populations [46] Furthermore, associations have also been found in Turkish male children [63], Pakistan population [64], and South African population [51]. However, neither Kobayashi *et al.* [27] nor Chimusa *et al.* [65] showed any association between rs3764880 and TB susceptibility

Ethnic differences in TLR polymorphisms may in part reflect the ethnic diversity of host TB susceptibility. Davila *et al.* found that around 30% of the Indonesian male subjects carried the A allele (rs3764880) associated with risk for TB, whereas this same allele was present in 78% of Russian patients [46]. 34.3% of the Turkish male children with pulmonary TB had the A allele associated with risk for TB, which is similar to Indonesian population [63]. The rs3764880 polymorphism of TLR8 was observed more than 16% among the healthy Chinese adult population by Cheng *et al.* [66].

The gene of TLR9 is located on chromosome 3p21.3. The total length of TLR9 gene is approximate 5 kb. Its coding gene has two exons, and the major coding region is in the second exon [52, 62]. TLR9 is an intracellular pathogen recognition receptor (PRR) that recognizes non-methylated cytosine-phosphate-guanine (CpG) motifs in bacterial DNA [52, 62]. Based on NCBI SNP database, twelve SNPs have been identified for TLR9 gene. Studies have been indicated certain race population with special genotype of TLR9 polymorphism might have higher risk for TB. Our results revealed that TLR9 rs352165, rs352167 and rs187084 allele were more frequently found in the patients population compared to the healthy controls population. Sanchez *et al.* found that TLR9 rs352165, rs352167 were not associated with TB risk in a Colombian population [37].

In previous study, a meta-analysis was performed to assess the association between seven extensively studied TLR9 polymorphisms (rs187084, rs352165, rs5743836,

rs5743842, rs352139, rs352140 and rs352167) and TB risk. The analysis revealed an association between certain TLR9 polymorphism and TB risk. The studies included Indians, Iranian and West African, Indonesians, Vietnamese, Chinese and Mexicans. The results showed that rs187084 and rs5743836 polymorphisms were not associated with TB risk, while the association between rs352139 polymorphism and TB risk may vary by race [67, 68].

Bharti *et al*/ found that rs187084 locus may be associated with susceptibility to TB in Indian population [68] The SNP rs187084 and rs5743836 SNPs located in the promoter are the most important and have been associated with various inflammatory diseases is located in the promoter of TLR9 gene [69–71]. Previous functional analyses have shown that both rs187084 and rs5743836 SNPs influence the transcription of TLR9 by regulation of promoter activity [69, 71, 72]. Some studies found that the rs187084 in TLR9 showed no association with TB in Vietnam and Iran [73, 74].

Our result revealed that rs5743836, rs164637, rs352139, rs352140, rs352143, and rs352162 allele may not be risk factors for susceptibility to pulmonary tuberculosis in Sudanese populations. On the other hands rs352139 has been strongly associated with susceptibility to TB in Indonesian [71] and Vietnamese populations [27, 72], African-Americans and in Mexican Amerindians. but not with Caucasians and African patients from Guinea-Bissau [30]. Sanders *et al*/ found that TLR9 rs5743836 and TLR9 rs352140 alleles have protective effect against meningococcal meningitis in Dutch children [75].

The rs5743836 in TLR9 showed a strong association with tuberculosis in African-Americans and Caucasians [31], while the association was not found in Vietnam [74] or Mexico population [73] and Chinese population [52]. Different variants of TLR9 rs352140 polymorphisms are described in the genome database [76] and in the Asian population: in Japan [77].

TLR9 rs352142 polymorphism was positively associated with meningeal TB, while variant *TWF2* rs352143 was associated with pulmonary TB in a Vietnamese cohort.[74] .

Recently, a number of studies indicated that TLR10 serves as a modulatory pattern-recognition receptor with mainly inhibitory properties on TLR2-derived immune responses, which are involved in the progression of TB [38, 39]. Our result revealed that TLR10 rs4129009 that was associated with TB susceptibility in Sudanese tuberculosis patients. Various studies found that Single-nucleotide polymorphisms in TLR10 were associated with susceptibility to tuberculosis in different ethnic groups. Ma *et al.* demonstrated that polymorphisms of TLR10 were significantly associated with TB in African and European Americans [38].

Bulat-Kardum *et al.* found that the rs11096957 AA genotype was associated with a predisposition to TB in the Caucasian population [78]. However, in the present study, we did not observe association between rs11096957 and risk of TB, no association was also found in Han Chinese population [79]. This inconsistent result is likely due to the ethnic difference

In conclusion our result indicates that the genotypes obtained for TLR1 rs5743557, TLR1 rs4833095, TLR1 rs5743596, TLR2 rs5743704, TLR2 rs5743708, TLR2 rs3804099, TLR4 rs4986790 ,TLR4 rs4986791, TLR6 rs5743810, TLR8 rs3764879, TLR8 rs3764880, TLR9 rs352165, TLR9 rs352167, TLR9 rs187084 and TLR10 rs4129009 have a significant role in the genetic susceptibility to tuberculosis in Sudanese population. Further studies on interaction of these polymorphisms and their effect on immune response to TB might lead to better understanding on the pathogenesis of disease.

Abbreviations

Tuberculosis ,TB ; *Mycobacterium tuberculosis* ,*M. tuberculosis*; Toll like Receptors, TLRs ;PCR-RFLP ,polymerase chain reaction and restriction fragment length polymorphism; SNPs, single nucleotide polymorphism ;HWE ,Hardy-Weinberg equilibrium.

Declarations

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Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate The present study was approved by the Ethics Committee of University of Khartoum, Khartoum, Sudan (5/2018). Written informed consents were obtained from all participants in the study

Consent to publish Not applicable.

Competing interests The authors declare that they have no competing interests.

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Tables

Table 1: Characteristics of the Study population

		Patients	Controls	p-value
Total number		160	220	
Mean age /yrs (range)		26 (11-70)	30 (11-70)	1.0
Gender (male/female)		111/49	65/155	0.0041
Occupation	Governmental employee	22 (13.8)	21 (9.55%)	0.05
	Workers	63 (39.4%)	44 (20%)	<0.0001
	Other job	40 (25%)	40 (18.3%)	0.58
	Jobless	3 (1.8%)	13 (5.9%)	<0.0001
	Housewife	13 (8.1%)	17 (7.7%)	0.03
	Student	19 (11.9%)	85 (38.6%)	0.15
BCG vaccination		112 (70.6%)	212 (96.8%)	0.63
Definite tuberculosis	Presence of MTB in sputum based on both smear and culture	92(57.5%)	0 (0%)	
	Presence of MTB in sputum specimen only by smear	109(68.1%)	0 (0%)	
	Presence of MTB in sputum specimen only by culture	47 (29.4%)	0 (0%)	
Hepatitis C test		Negative	Negative	
Hepatitis B Ag test		Negative	Negative	
HIV1, 2 test		Negative	Negative	
Nasopharyngeal swab for (COVID-19)		Negative	Negative	

unpaired t-test

Fisher exact test

Table 2: PCR primers and restriction enzymes for genotyping the different Single Nucleotide Polymorphisms

Gene(s)	Rs number	Primer sequence(s) (5'→3')	Chromosome position	Gene location	Reference
TLR 1					
T/C	rs5743604	F: GAAGAGCAGTCCCAATACCACCAT	chr4:38801285	Intron 3	[52,53, 56,62]
		R: GGCCCCAAGCTTCCCCAAAATTA			
C/G	rs5743611	F: GCATCTTCCATTTTGCCATTATCTTC	chr4:38798593	exon 4	[52,53, 56,62]
		R: CCAATTCCTGGTTGAATTTGAAAACA			
G/T	rs5743618	F: TCCTCATGACTCTTTTCTGC	chr4:38797027	Promoter	[52,53, 56,62]
		R: GAGGCTTGTCCCTTGCTCCAC			
A/G	rs76600635	F: GCATCTTCCATTTTGCCATTATCTTC	chr4: 38800323	intron	[52,53, 56,62]
		R: CCAATTCCTGGTTGAATTTGAAAACA			
A/G	rs5743551	F: CCGAGATGTTCCCAGCACAG	chr4: 38806033	Promoter	[52,53, 56,62]
		R :CTGCTTTGCTTGTGCCTCTT			
C/T	rs5743557	F:ACGTTGGATGGAGTGTGCTTCAGCAAAAC	chr4:38806827	near- gene-5	[52,53, 56,62]
		R:ACGTTGGATGTCTTGCCTTTTCATCCATCC			
A/G	rs5743565	F: ACGTTGGATGAGCTGAACAGCAGCATTGCC	chr4:38805983	exon 2	[52,53, 56,62]
		R: ACGTTGGATGTTCTCTTCACCTAATCCCGC			
C/G	rs5743566	F: ACGTTGGATGAAGGACTAGCTAGTGGAAG	chr4:38805942	exon 2	[52,53, 56,62]
		R: ACGTTGGATGAGTTACTCCCGGAGGCAATG			
A/G	rs5743580	F:ACGTTGGATGAACATCCAGAGTGACTCAGC	chr4:38804405	intron	[52,53, 56,62]
		R:ACGTTGGATGTGGCTATGGTAAGCTTCTC			
C/T	rs5743594	F:ACGTTGGATGGCTACTCACACAAGGAGCAA	chr4:38802751	intron	[52,53, 56,62]
		R:ACGTTGGATGGGGTTAGGATTTCAACATAAG			
G/A	rs4833095	F:ACGTTGGATGCTGGAGGATCCTAATGAAAG	chr4:38799710	exon 4	[52,53, 56,62]
		R:ACGTTGGATGCCTAAGTATTCTGGCGAAAC			
T/C	rs5743595	F:ACGTTGGATGGGAAATTTCCGGGTCTTTCA	chr4:38802644	intron	[52,53, 56,62]
		R:ACGTTGGATGGCAAGGAAGATGATGCAGAC			
C/T	rs5743596	F:ACGTTGGATGTGCTCAGGGTCTTCATGAAC	chr4:38802528	exon 3	[52,53, 56,62]
		R:ACGTTGGATGGGTGCCCAATATGCCTTTGT			
TLR 2					
C/T	rs1816702	F: ACGTTGGATGGTGTGAGCCTTACTAAAGGT	chr4:154609523	intron	[52,53, 56,62]

R: ACGTTGGATGCTTTGATTCTCTAGCGCTG					
C/A	rs5743704	F: ACGTTGGATGTGCATCATAGCAGATGTTCC	chr4:154625951	exon 3	[52,53, 56,62]
R: ACGTTGGATGTGAAAATGATGTGGGCCTG					
G/A	rs5743708	F:ACGTTGGATGCCAGGTAGGTCTTGGTGTTCC	chr4:154626317	exon 3	[52,53, 56,62]
R:ACGTTGGATGTTCTTCTGGAGCCCATTGAG					
T/C	rs7656411	F:ACGTTGGATGCCTTTAAATTACTGTGTATC	chr4:154627655	near gene-3	[52,53, 56,62]
R:ACGTTGGATGGTACATGTGAGCTAAATAG					
C/T	rs11938228	F:ACGTTGGATGTAGGGACATGCCCATATAGG	chr4:154621946	near-gene-5	[52,53, 56,62]
R:ACGTTGGATGTAGTGTGTTGCTGGTATAGG					
G/A	rs893629	F:ACGTTGGATGACATCACCTGAAACACACCG	chr4:154604968	near-gene-5	[52,53, 56,62]
R:ACGTTGGATGGATTCATGAGCCTGAGTTCCG					
A/G	rs1898830	F:ACGTTGGATGGATCCCCTATTTTCTAGCAC	chr4:154608453	intron	[52,53, 56,62]
R: ACGTTGGATGAAAACCTGGAAAAGGAATAG					
C/T	rs121917864	F:CACGTGAGTCTGAGTTTC	chr4:154626088	exon 3	[52,53,56,62]
R:GTCTGGTTCAAGAAGTCATACCCCAACCCA AGA GAG'					
T/A	rs4696480	F : ACGTTGGATGCTCACCATGTGATGCTTTCC	chr4:154607126	intron	[52,53, 56,62]
R : ACGTTGGATGGGGAAGTCCAAGATTGAAGG					
T/C	rs3804099	F:ACGTTGGATGCTGCTTCATATGAAGGATCAG	chr4:154624656	exon 3	[52,53, 56,62]
R : ACGTTGGATGGATCTACAGAGCTATGAGCC					
T/C	rs5743699	F :'ATTTGAACTTATCCA GCACACGAAT	chr4:154625291	exon 3	[52,53, 56,62]
R :TCCAGTGTCTTGGAATGCA					
T/C	rs3804100	F : ACGTTGGATGTTGAACTTATCCAGCACACG	chr4:154625409	exon 3	[52,53, 56,62]
R : ACGTTGGATGTTCCAGTGTCTTGGAATGC					
TLR 4					
C/T	rs7869402	F : ACGTTGGATGTTTAGGGAGACACAGATGGC	Chr9:120478032	exon 3	[52,53, 56,62]
R : ACGTTGGATGACCTTCACACGTAGTTCTCC					
G/A	rs1927907	F: ACGTTGGATGTTTTTCAAACAAGAAGTAG	Chr9:120472764	intron	[52,53, 56,62]
R: ACGTTGGATGGGGTATCCAGTGGATTGAAG					
C/T	rs1927911	F: ACGTTGGATGAGACCTTCCTTAGTCATGGC	Chr9:120470054	intron	[52,53, 56,62]
R: ACGTTGGATGCATCACTTTGCTCAAGGGTC					
T/C	rs1927914	F : ACGTTGGATGGTGCTTGGAGGATATTACAG	Chr9:120464725	near-gene-5	[52,53, 56,62]
R : ACGTTGGATGGAACCTGATTTAAACAGGA					

A/G	rs6478317	F:ACGTTGGATGTCTATCCCCAAGATCGGTTCC	Chr9:120480210	near-gene-5	[52,53, 56,62]
		R:ACGTTGGATGAAGAGGCTAGAAGAAGATAG			
A/G	rs55912718	F: AATTCACCAAGCCCAGGCAGAG	Chr9:120466480	near-gene-5	[52,53, 56,62]
		R: GAGGAAGTGAAAGCGGCAACCT			
G/A	rs5030719	F: GCTGAGAACTTGACCTTCCTGGAC	Chr9:120475936	near-gene-5	[52,53, 56,62]
		R: AGAACCTGGAGGGAGTTCAGACAC			
C/T	rs10759931	F: ACCTCAGTGGGCTCTGGGGTAG	Chr9:120464147	near-gene-5	[52,53, 56,62]
		R: CCCTTCCTCTGAACCACCTCCT:			
A/C	rs10759933	F :ACGTTGGATGGGTAATAAAATATCCAATATCG	Chr9:120470372	intron	[52,53, 56,62]
		R : ACGTTGGATGTGCTCATCTTCTCTGTATCC			
T/C	rs2770150	F: ACGTTGGATGCACTCAATCATTTACTGACC	Chr9:120463139	near-gene-5	[52,53, 56,62]
		R: ACGTTGGATGACACATGGTCTGCCTTCTGG			
C/T	rs1554973	F: TGAAGCCATGAATCATTAAGCCAATA	Chr9:120480812	intron	[52,53, 56,62]
		R: TCCAAAAGCCACGCTACTCAAA			
C/A	rs11536878	F:ACGTTGGATGTTCTTGACTACCCACCACAG	Chr9:120471553	intron	[52,53, 56,62]
		R:ACGTTGGATGGCGACATATAACAGTAGGTG			
A/C	rs11536879	F: CCTGTTGGGGTCAGAAGACCTG	Chr9:120472211	intron	[52,53, 56,62]
		R:CATTTAATCCAGCAGAATAAGTTTCATCATT			
G/C	rs7873784	F: ACGTTGGATGGCTCTAAAGATCAGCTGTAT	Chr9:120478936	exon 4	[52,53, 56,62]
		R: ACGTTGGATGGGTACCCTCTTAACAAAATG			
G/C	rs11536889	F :GTTTCCTGTTGGGCAATG CT	Chr9:120478131	near-gene-3	[52,53, 56,62]
		R :CATTAAATCCAGCACATTGTTTTCTC			
A/G	rs4986790	F: TCTTGCTGGAGTATTCCTATGA	Chr9:120475302	exon 4	[52,53, 56,62]
		R: GCCACAACCTGCTCTTGC			
C/T	rs4986791	F:GGTTGCTGTTCTCAAAGTGATTTTGGGAGAA	Chr9:120475602	exon 4	[52,53, 56,62]
		R:ACCTGAAGACTGGAGAGTGAGTTAAATGCT			
G/A	rs11536897	F: TTCTGCATAAGTGTGTTTATTCAAGCAAA	Chr9:120480010	exon 4	[52,53, 56,62]
		R: TCCAGAGGATTTAAAGAGCTAAAAAGAGG			
C/A	rs11536898	F: TTCTGCATAAGTGTGTTTATTCAAGCAAA	Chr9:120480210	exon 4	[52,53, 56,62]
		R: TCCAGAGGATTTAAAGAGCTAAAAAGAGG			
TLR 6					
T/C	rs3796508	F: ACGTTGGATGGTTGCAAAAGATATCCTGCC	chr4: 38830736	exon 1	[52,53, 56,62]
		R: ACGTTGGATGGCAGGGCCTTGAAATCATTG			

C/T	rs5743810	F: ACGTTGGATGATTTTTATCAGAACTCACC R: ACGTTGGATGAGGCATTTCCAAGTCGTTTC	chr4: 38830350	exon 1	[52,53, 56,62]
T/C	rs5743831	F: GTTGGCTTGAGCACCCAAAATG R: CTTCTGCCTAACCCCGTGCCT	chr4:38827414	intron	[52,53, 56,62]
T/C	rs1039559	F: ACGTTGGATGGCAAGCAGCAGACACATCAA R: ACGTTGGATGCTCAGCCTTTTTTCTCCAC	chr4:38831596	near-gene-5	[52,53, 56,62]
T/C	rs6531670	F: CCACGAGTGCCACATATTTAC R: CAGTGTCAGTGGCATTGGGTTC	chr4: 38835022	intron	[52,53, 56,62]
C/G	rs5743788	F:ACGTTGGATGGACACTGCAAGAGAGTGAAA R:ACGTTGGATGCAAGTCCCTATCATATATGG	chr4:38833207	Promoter	[52,53, 56,62]
C/T	rs5743794	F: TGAGTTGCCTTTGCTCGTTTGTC R: AGCCAGATAACTGACACCACCTAACA	chr4: 38832727	intron	[52,53, 56,62]
TLR 8					
G/A	rs4830805	F: ACGTTGGATGAAGGGAACGTGGAAAATCCG R: ACGTTGGATGATCAGCAGAGACCTGATAGC	ChrX: 12927759	intron	[52,53, 56,62]
C/T	rs4830808	F: ACGTTGGATGGGTAAATGGGTCTCCATTTAG R: ACGTTGGATGTCCCACTGGAATTGTTTAGG	ChrX:12932334	intron	[52,53, 56,62]
C/A	rs3747414	F: ACGTTGGATGGGTCTTAGTTTCAAGTGCGG R: ACGTTGGATGAGTCAGTAGTCTGAAGCACC	ChrX: 12939412	exon 2	[52,53, 56,62]
A/G	rs3761624	F: ACGTTGGATGTTGGTTTTCTCCACTCCTG R: ACGTTGGATGCCCTGGCCACAAGAATAAAG	ChrX: 12923681	near-gene-5	[52,53, 56,62]
C/T	rs1548731	F: ACGTTGGATGACCCAAAGAAGTCCATGAGG R: ACGTTGGATGGAGTGACCTTGAAAACATTC	ChrX: 12927947	intron	[52,53, 56,62]
A/T	rs2109134	F: ACGTTGGATGCCCAGATGAACTCAGAGATG R: ACGTTGGATGAAGTGCATCATAGTGTGCGC	ChrX: 12927186	intron	[52,53, 56,62]
A/G	rs3788935	F: ACGTTGGATGGTCTAGTATCTATGTCAAGC R: ACGTTGGATGGTGTGGAGAAAAGTGAAGGC	ChrX: 12922659	near-gene-5	[52,53, 56,62]
G/A	rs1013150	F: ACGTTGGATGATCACTCAGGGCAGTGTAAG R: ACGTTGGATGTTACTAGGAAAAAGATGAG	ChrX: 12932441	intron	[52,53, 56,62]
C/T	rs5744068	F: ACGTTGGATGTCATTTCTTGCTAGCTGCC R: ACGTTGGATGCCCAACAAGGAATGCAAG	ChrX: 12935058	intron	[52,53, 56,62]
G/C	rs3764879	F: GTGTGTGTCTGATTTGGGTTG R: TTTCTAGGCTCACACCATTTG	ChrX:12924697	intron	[52,53, 56,62]
C/T	rs5744080	F: ACGTTGGATGGCTGACAAATTTGGAGTTGC	ChrX:12937804	exon 2	[52,53, 56,62]

R: ACGTTGGATGGTAGGGAGCTTGGCAGTTTG					
A/G	rs3764880	F: ACGTTGGATGGCTAAAGAAATAGAAGTGGC	ChrX:12924826	exon 1	[52,53, 56,62]
R: ACGTTGGATGCTGCTGCAAGTTACGGAATG					
C/T	rs17256081	F: ACGTTGGATGGCACTAAAATTTTACAATGC	ChrX: 12926045	intron	[52,53, 56,62]
R: ACGTTGGATGATGTGTTCATTGGCCCAGTTG					
C/T	rs5741883	F: ACGTTGGATGAAGCGAGCATCTTTCTCCTG	ChrX: 12924221	near-gene-5	[52,53, 56,62]
R: ACGTTGGATGACAATGAACACTCATTGAGC					
G/C	rs2407992	F: ACGTTGGATGGACTCGCTGGCAAATTAAGG	ChrX: 12939112	exon 2	[52,53, 56,62]
R: ACGTTGGATGGACACGTCTGGATTTATCCC					
G/A	rs178998	F: ACGTTGGATGCCTAAACTGTATGCATTGCC	ChrX: 12917787	near-gene-5	[52,53, 56,62]
R" ACGTTGGATGTGGGCCTTCCTGTAGAAGAC					
TLR 9					
A/G	rs5743836	F: GAGGGTGACATGGGAGCAGAGA	Chr3 :52260782	Promoter	[[52,53, 56,62]
R:CTGTGTAGCCCCTGGGCATTCT					
G/A	rs164637	F:CCCTGCAGGATGACCTCTCTTT	Chr3: 52265215	exon 2	[52,53, 56,62]
R:AGGCACCCACCATCTGGATGTA					
T/C	rs352139	F:CTGTGGACATCGATATCGGTGT	Chr3: 52258372	intron	[52,53, 56,62]
R:AAGCTTCGCTGCGGCAGAAACCCTGT					
C/T	rs352140	F:TCTAGACATCATGCTGGCCATGACC	Chr3: 52256697	intron	[52,53, 56,62]
R: CAGAGCCACTCAACAGTGGACT					
T/C	rs352143	F: CCCTGCAGGATGACCTCTCTTT	Chr3: 52264907	exon 2	[52,53, 56,62]
R: AGGCACCCACCATCTGGATGTA					
T/C	rs352162	F: CCCTGCAGGATGACCTCTCTTT	Chr3: 52252969	intron	[52,53, 56,62]
R: AGGCACCCACCATCTGGATGTA					
A/G	rs352165	F: CCCTGCAGGATGACCTCTCTTT	Chr3: 52242902	intron	[52,53, 56,62]
R: AGGCACCCACCATCTGGATGTA					
T/C	rs352167	F: CCCTGCAGGATGACCTCTCTTT	Chr3: 52238656	intron	[52,53, 56,62]
R: AGGCACCCACCATCTGGATGTA					
A/G	rs187084	F:CTGTGGACATCGATATCGGTGT	Chr3: 52261031	Promoter	[52,53, 56,62]
R:AAGCTTCGCTGCGGCAGAAACCCTGT					
TLR 10					
A/G	rs4129009	F:CCAGAATGAGTGGTGCCATT	chr4:38773268	exon 4	[52,53, 56,62]
R: GTATGTGGTCCCCAACTTCCC					
A/G	rs7694115	F: ACGTTGGATGCTATAGGTTGCCTCAAACAG	chr4:38779094	intron	[52,53, 56,62]

R:ACGTTGGATGCAGCAGAAGATTTAGAGTCC					
A/C	rs10856839	F: ACGTTGGATGGTTTCTGATGAGTCTCATTG	chr4:38777236	exon 4	[52,53, 56,62]
R: ACGTTGGATGGGGTTTTGAGCTCATCTTC					
T/A	rs11466645	F: GATGAGGAAATTGAAGGATCT3'	chr4:38778203	exon 4	[52,53, 56,62]
R: CACTGCTACTTCCCCAGTGC3'					
G/A	rs4274855	F: ACGTTGGATGTCCTCTCTGAGAATCCTGAC	chr4:38777471	exon 3	[52,53, 56,62]
R: ACGTTGGATGTTGGCTGAGAAGTCTCCAAG					
A/C	rs11096955	F:ACGTTGGATGTGAGAGTTTTCAAGTGAGGC	chr4:38776107	exon 4	[52,53, 56,62]
R:ACGTTGGATGCCAATAATATCTTAACAGACG					
G/T	rs11096956	F:ACGTTGGATGCCTGACAATATCAAATGCAC	chr4:38776180	exon 4	[52,53, 56,62]
R:ACGTTGGATGAATATTGGAATTTCGTAGG					
A/C	rs11096957	F: ACGTTGGATGTAAGCAATAGAACCGATGTC	chr4:38776491	exon 4	[52,53, 56,62]
R: ACGTTGGATGGGCAAAAGCCAATTTGTAAG					
G/A	rs7698870	F: ACGTTGGATGTACCACGGAATGAACAGAG	chr4:38781459	intron	[52,53, 56,62]
R: ACGTTGGATGGATCTCTTCTGAATGACCTC					
T/C	rs10776483	F: ACGTTGGATGATGGAATGGGTTCCAGTAAG	chr4:38775040	exon 4	[52,53, 56,62]
R: ACGTTGGATGTGAATTCTACTTTGCCACC					

Table 3: Genotype distributions and Hardy Weinberg Equilibrium in Sudanese tuberculosis patients and healthy controls

Genotype	Tuberculosis patients N=160(%)	HWE of patient population*	Control N=220(%)	HWE of control population*
TLR 1- rs5743604				
TT	65(40.62)	<0.01	70(31.82)	<0.01
TC	22(13.75)		52(23.63)	
CC	73(45.63)		98(44.55)	
TLR 1- rs5743611				
CC	122(76.25)	<0.01	193(87.72)	<0.01
CG	26(16.25)		19(8.64)	
GG	12(7.5)		8(3.64)	
TLR 1- rs5743618				
GG	148(92.5)	<0.01	201(91,36)	<0.01
GT	8(5.00)		17(7.73)	
TT	4(2.5)		2(0.91)	
TLR 1- rs76600635				
AA	152(95.00)	<0.01	190(86.36)	<0.01
AG	7(4.38)		30(13.64)	
GG	1(0.62)		0(0.00)	
TLR 1- rs5743551				
AA	130(81.25)	<0.01	197(89.55)	<0.01
AG	15 (9.37)		18(8.18)	
GG	15(9.37)		5(2.27)	
TLR 1- rs5743557				
CC	60(37.5)	<0.01	84(38.18)	<0.01
CT	71(44.38)		70 (31.82)	
TT	29(18.12)		66(30.00)	
TLR 1- rs5743565				
AA	120(75.00)	<0.01	116(52.73)	<0.01
AG	26(16.25)		65(29.54)	
GG	14(8.75)		39(17.73)	
TLR 1- rs5743566				
CC	135(84.38)	<0.01	218(99.09)	<0.01
CG	8(5.00)		2 (0.91)	
GG	17(10.62)		0(0.00)	
TLR 1- rs5743580				
AA	79(49.38)	<0.01	81(36.82)	<0.01
AG	39(24.37)		44(20.00)	

GG	42(26.25)		95(43.18)	
TLR 1- rs5743594				
CC	70(43.75)	<0.01	76(34.55)	<0.01
CT	22(14.37)		47(21.36)	
TT	68(42.5)		97(44.09)	
TLR 1- rs4833095				
GG	128(80.00)	<0.01	198(90.00)	<0.01
GA	22(13.75)		18(8.18)	
AA	10(6.25)		4(1.82)	
TLR 1- rs5743595				
TT	122(76.25)	<0.01	212(96,36)	<0.01
TC	10(6.25)		8(3.64)	
CC	28(17.5)		0(0.00)	
TLR 1- rs5743596				
CC	75(46.87)	<0.01	72(32.72)	<0.01
CT	22(13.75)		50(22.73)	
TT	63(39.38)		98(44.55)	
TLR 2- rs1816702				
CC	124(77.5)	<0.01	190(86.36)	<0.01
CT	24(15.0)		22(10.00)	
TT	12(7.5)		8(3.64)	
TLR 2- rs5743704				
CC	144(90.0)	<0.01	212(96,36)	<0.01
CA	12(7.5)		8(3.64)	
AA	4(2.5)		0(0.00)	
TLR 2- rs5743708				
GG	154(96.25)	<0.01	197(89.55)	<0.01
GA	4(2.5)		23(10.45)	
AA	2(1.25)		0(0.00)	
TLR 2- rs7656411				
TT	128(80.00)	<0.01	200(90.91)	<0.01
TC	16 (10.00)		20(9.09)	
CC	16(10.00)		0(0.00)	
TLR 2- rs11938228				
CC	56(35.00)	<0.01	75(34.09)	<0.01
CT	75(46.88)		78(35.45)	

TT	29(18.12)		67(30.45)	
TLR 2- rs893629				
GG	118(73.75)	<0.01	104(47.27)	<0.01
GA	26(16.25)		70(31.82)	
AA	16(10.00)		46(20.91)	
TLR 2- rs1898830				
AA	150(93.75)	<0.01	216(98.18)	<0.01
AG	3(1.88)		4(1.82)	
GG	7(4.37)		0(0.00)	
TLR 2- rs121917864				
CC	74(46.25)	<0.01	76(34.55)	<0.01
CT	46(28.75)		47(21.36)	
TT	40(25.0)		97(44.09)	
TLR 2- rs4696480				
TT	68(42.50)	<0.01	75(34.09)	<0.01
TA	26(16.25)		47(21.36)	
AA	66(41.25)		98(44.55)	
TLR 2- rs3804099				
TT	124(77.5)	<0.01	194(88.18)	<0.01
TC	24(15.0)		20(9.09)	
CC	12(7.5)		6(2.73)	
TLR 2- rs5743699				
TT	144(90.0)	<0.01	216(98.18)	<0.01
TC	10(6.25)		4(1.82)	
CC	6(3.75)		0(0.00)	
TLR 2- rs3804100				
TT	66(41.25)	<0.01	80(36.36)	<0.01
TC	26(16.25)		48(21.82)	
CC	68(42.5)		92(41.82)	
TLR 4 - rs7869402				
CC	128(80.00)	<0.01	190(86.36)	<0.01
CT	22(13.75)		22(10.00)	
TT	10(6.25)		8(3.64)	
TLR 4 - rs1927907				
GG	144(90.00)	<0.01	210(95.45)	<0.01
GA	12(7.5)		10(4.55)	

AA	4(2.5)		0(0.00)	
TLR 4 - rs1927911				
CC	70(43.75)	<0.01	74(33.63)	<0.01
CT	22(13.75)		48(21.82)	
TT	68(42.5)		98(44.55)	
TLR 4 - rs1927914				
TT	126(78.75)	<0.01	188(85.45)	<0.01
TC	22(13.75)		20(9.09)	
CC	12(7.5)		12(5.45)	
TLR 4 - rs6478317				
AA	140(87.5)	<0.01	182(82.73)	<0.01
AG	16(10.0)		38(17.27)	
GG	4(2.5)		0(0.00)	
TLR 4 - rs55912718				
AA	152(95.0)	<0.01	190(86.36)	<0.01
AG	4(2.5)		30(13.64)	
GG	4(2.5)		0(0.00)	
TLR 4 - rs5030719				
GG	124(77.5)	<0.01	202(91.82)	<0.01
GA	20 (12.5)		18(8.18)	
AA	16(10.0)		0(0.00)	
TLR 4 - rs10759931				
CC	60(37.5)	<0.01	70(31.82)	<0.01
CT	73(45.63)		78(35.45)	
TT	27(16.87)		72(32.73)	
TLR 4 - rs10759933				
AA	122(76.25)	<0.01	108(49.09)	<0.01
AC	24(15.00)		68(30.91)	
CC	14(8.75)		44(20.00)	
TLR 4 - rs2770150				
TT	150(93.75)	<0.01	216(98.18)	<0.01
TC	3(1.88)		4(1.82)	
CC	7(4.37)		0(0.00)	
TLR 4 - rs1554973				
CC	74(46.25)	<0.01	76(34.55)	<0.01
CT	46(28.75)		48(21.82)	
TT	40(25.00)		96(43.63)	

TLR 4 - rs11536878				
CC	72(43.13)	<0.01	74(33.63)	<0.01
CA	40(14.37)		48(21.82)	
AA	48(42.5)		98(44.55)	
TLR 4 - rs11536879				
AA	120(75.00)	<0.01	192(87.27)	<0.01
AC	28(17.5)		22(10.00)	
CC	12(7.5)		6(2.73)	
TLR 4 - rs7873784				
GG	144(90.00)	<0.01	212(96.36)	<0.01
GC	12(7.5)		8(3.64)	
CC	4(2.5)		0(0.00)	
TLR 4 - rs11536889				
GG	70(43.75)	<0.01	74(33.63)	<0.01
GC	22(13.75)		48(21.82)	
CC	68(42.5)		98(44.55)	
TLR 4 - rs4986790				
AA	128(80.00)	<0.01	194(88.18)	<0.01
AG	20(12.5)		18(8.18)	
GG	12(7.5)		8(3.64)	
TLR 4 - rs4986791				
CC	70(43.75)	<0.01	90(40.91)	<0.01
CT	22(13.75)		44(20.00)	
TT	68(42.5)		86(39.09)	
TLR 4 - rs11536897				
GG	124(77.5)	<0.01	192(87.27)	<0.01
GA	20(12.5)		24(10.91)	
AA	16(10.0)		4(1.82)	
TLR 4 - rs11536898				
CC	152(95.00)	<0.01	216(98.18)	<0.01
CA	6(3.75)		4(1.82)	
AA	2(1.25)		0(0.00)	
TLR 6- rs3796508				
TT	146(91.25)	<0.01	202(91.82)	<0.01
TC	10(6.25)		16(7.27)	
CC	4(2.5)		2(0.91)	

TLR 6- rs5743810				
CC	130(81.25)	<0.01	203(92.73)	<0.01
CT	15 (9.37)		15(7.27)	
TT	15(9.37)		2(0.91)	
TLR 6- rs5743831				
TT	60(37.5)	<0.01	70(31.82)	<0.01
TC	73(45.62)		78(35.45)	
CC	27(16.88)		72(32.73)	
TLR 6- rs1039559				
TT	70(43.75)	<0.01	100(45.45)	<0.01
TC	24(15.00)		48(21.82)	
CC	66(41.25)		72(32.73)	
TLR 6- rs6531670				
TT	74(46.25)	<0.01	102(46.36)	<0.01
TC	22(13.75)		40(18.18)	
CC	64(40.00)		78(35.45)	
TLR 6- rs5743788				
CC	128(80.00)	<0.01	186(84.55)	<0.01
CG	20(12.5)		30(13.63)	
GG	12(7.5)		4(1.82)	
TLR 6- rs5743794				
CC	122(76.25)	<0.01	216(98,18)	<0.01
CT	36(22.50)		4(1.82)	
TT	2(1.25)		0(0.00)	
TLR 8- rs4830805				
GG	75(46.88)	<0.01	88(40.00)	<0.01
GA	22(13.75)		42(19.09)	
AA	63(39.37)		90(40.91)	
TLR 8- rs4830808				
CC	128(80.00)	<0.01	194(88.18)	<0.01
CT	22(13.75)		22(10.00)	
TT	10(6.25)		4(1.82)	
TLR 8- rs3747414				
GG	150(93.75)	<0.01	216(98,18)	<0.01
GT	8(5.00)		4(1.82)	
TT	2(1.25)		0(0.00)	

TLR 8- rs3761624		<0.01		<0.01
CC	146(91.25)		200(90.91)	
CA	9(5.63)		20(9.09)	
AA	5(3.12)		0(0.00)	
TLR 8- rs1548731		<0.01		<0.01
CC	131(81.88)		204(92.73)	
CT	14 (8.75)		16(7.27)	
TT	15(9.37)		0(0.00)	
TLR 8- rs2109134		<0.01		<0.01
AA	58(36.25)		75(34.09)	
AT	73(45.63)		78(35.45)	
TT	29(18.12)		67(30.45)	
TLR 8- rs3788935		<0.01		<0.01
AA	69(43.13)		75(34.09)	
AG	23(14.37)		47(21.36)	
GG	68(42.5)		98(44.55)	
TLR 8- rs1013150				
GG	130(81.25)	<0.01	204(92.73)	<0.01
AG	14 (8.75)		16(7.27)	
AA	16(10.0)		0(0.00)	
TLR 8- rs5744068				
CC	52(32.5)	<0.01	80(36.36)	<0.01
CT	76(47.5)		73(33.18)	
TT	32(20.0)		67(30.45)	
TLR 8- rs3764879				
GG	80(50.0)	<0.01	70(31.82)	<0.01
GC	40(25.0)		60(27.27)	
CC	40(25.0)		90(40.91)	
TLR 8- rs5744080	70(43.75)		83(37.73)	
CC	20(12.5)	<0.01	47(21.36)	<0.01
CT	70(43.75)		90(40.91)	
TT				
TLR 8- rs3764880				
AA	155(96.87)	<0.01	218(99.09)	<0.01
AG	1(0.63)		2(0.91)	
GG	4(2.5)		0(0.00)	
TLR 8- rs17256081				

CC	86(53.75)	<0.01	210(95.45)	<0.01
CT	52(32.5)		10(4.55)	
TT	22(13.75)		0(0.00)	
TLR 8- rs5741883				
CC	75(46.88)		216(98.2)	
CC	8(5.00)	<0.01	4(1.8)	<0.01
CT	77(48.12)		0(0.0)	
TT				
TLR 8- rs2407992				
GG	136(85.00)	<0.01	170(77.27)	<0.01
GC	10(6.25)		40(18.18)	
CC	14(8.75)		10(4.55)	
TLR 8- rs178998				
GG	98(61.25)			
GG	22(13.75)	<0.01	198(90.00)	<0.01
GA	40(25.00)		20(9.09)	
AA			2(0.91)	
TLR 9- rs5743836				
AA	69(43.13)	<0.01	75(34.09)	<0.01
AG	23(14.37)		47(21.36)	
GG	68(42.5)		98(44.55)	
TLR 9- rs164637				
GG	126(78.75)	<0.01	192(87.27)	<0.01
GA	22(13.75)		20(9.09)	
AA	12(7.5)		8(3.64)	
TLR 9- rs352139				
TT	146(91.25)	<0.01	213(96.82)	<0.01
TC	10(6.25)		7(3.18)	
CC	4(2.5)		0(0.00)	
TLR 9- rs352140				
CC	155(96.88)	<0.01	197(89.55)	<0.01
CT	4(2.5)		23(10.45)	
TT	1(0.62)		0(0.00)	
TLR 9- rs352143				
TT	129(80.63)	<0.01	202(91.82)	<0.01
TC	15 (9.37)		18(8.18)	
CC	16(10.0)		0(0.00)	
TLR 9- rs352162				
TT	58(36.25)	<0.01	75(34.09)	<0.01

TC	73(45.63)		78(35.45)	
CC	29(18.12)		67(30.45)	
TLR 9- rs352165				
AA	120(75.00)	<0.01	106(48.18)	<0.01
AG	26(16.25)		70(31.82)	
GG	14(8.75)		44(20.00)	
TLR 9- rs352167				
TT	152(95.00)	<0.01	216(98.18)	<0.01
TC	1(0.63)		4(1.82)	
CC	7(4.37)		0(0.00)	
TLR 9- rs187084				
AA	76(47.5)	<0.01	74(33.64)	<0.01
AG	44(27.5)		49(22.27)	
GG	40(25.0)		97(44.09)	
TLR 10- rs4129009				
AA	75(46.88)		88(40.00)	
AG	22(13.75)		42(19.09)	
GG	63(39.37)		90(40.91)	
TLR 10- rs7694115				
AA	128(80.00)	<0.01	194(88.18)	<0.01
AG	22(13.75)		22(10.00)	
GG	10(6.25)		4(1.82)	
TLR 10- rs10856839				
AA	150(93.75)	<0.01	216(98.18)	<0.01
AC	8(5.00)		4(1.82)	
CC	2(1.25)		0(0.00)	
TLR 10- rs11466645				
TT	146(91.25)	<0.01	200(90.91)	<0.01
TA	9(5.63)		20(9.09)	
AA	5(3.12)		0(0.00)	
TLR 10- rs4274855				
GG	131(81.88)	<0.01	204(92.73)	<0.01
GA	14 (8.75)		16(7.27)	
AA	15(9.37)		0(0.00)	
TLR 10- rs11096955				
AA	58(36.25)	<0.01	75(34.09)	<0.01

AC	73(45.63)		78(35.45)	
CC	29(18.12)		67(30.45)	
TLR 10- rs11096956		<0.01		<0.01
GG	69(43.13)		75(34.09)	
GT	23(14.37)		47(21.36)	
TT	68(42.5)		98(44.55)	
TLR 10- rs11096957				
AA	130(81.25)	<0.01	204(92.73)	<0.01
AC	14 (8.75)		16(7.27)	
CC	16(10.0)		0(0.00)	
TLR 10- rs7698870				
GG	52(32.5)	<0.01	80(36.36)	<0.01
GA	76(47.5)		73(33.18)	
AA	32(20.0)		67(30.45)	
TLR 10- rs10776483				
TT	80(50.0)	<0.01	70(31.82)	<0.01
TC	40(25.0)		60(27.27)	
CC	40(25.0)		90(40.91)	

Table 4: Allele frequencies of tuberculosis patients in comparison to a matching healthy control population

Genotype	Tuberculosis patients N=160(%)	Control N=220(%)	P-value for Association	OR (95% CI)
TLR 1- rs5743604 T/C				
T-allele	152	192	0.352	1.03 (0.79-1.63)
C-allele	168	239		
TLR 1- rs5743611C/G				
C-allele	270	405	0.674	1.09 (0.75-1.45)
G-allele	50	35		
TLR 1- rs5743618G/T				
G-allele	304	419	0.456	1.18 (0.75-1.74)
T-allele	24	21		
TLR 1- rs76600635A/G				
A-allele	311	410	0.182	1.20 (0.80-1.26)
G-allele	9	30		
TLR 1- rs5743551A/G				
A-allele	275	412	0.472	1.26 (0.66-2.24)
G-allele	45	28		
TLR 1- rs5743557C/T				
C-allele	191	238	<0.0001	1.39 (0.77- 2.40)
T-allele	129	202		
TLR 1- rs5743565A/G		297	0.672	
A-allele	266	143		1.33 (0.67-2.17)
G-allele	54			
TLR 1- rs5743566C/G		438	0.344	
C-allele	278	2		1.22 (0.85-1.45)
G-allele	42			
TLR 1- rs5743580A/G		206	0.455	
A-allele	197	234		1.15 (0.76-1.76)
G-allele	123			
TLR 1- rs5743594C/T		199	0.621	
C-allele	162	241		1.23 (0.78-1.65)
T-allele	158			
TLR 1- rs4833095 G/A		414	<0.0001	
G-allele	278	26		1.17 (0.77-1.89)
A-allele	42			
TLR 1- rs5743595 T/C		432	0.662	

T-allele	254	8		1.05 (0.64- 1.78)
C-allele	66			
TLR 1- rs5743596 C/T		194	<0.0001	
C-allele	172	246		1.00 (0.54-1.45)
T-allele	148			
TLR 2- rs1816702C/T		402	0.705	
C-allele	272	38		1.57 (1.40-2.25)
T-allele	48			
TLR 2- rs5743704C/A		432	<0.0001	
C-allele	300	8		1.15 (0.75-1.45)
A-allele	20			
TLR 2- rs5743708 G/A			<0.0001	
G-allele	312	417		1.23 (0.95-1.45)
A-allele	8	23		
TLR 2- rs7656411 T/C		420	0.234	
T-allele	272	20		1.97 (0.75-2.12)
C-allele	48			
TLR 2- rs11938228 C/T		228	0.648	
C-allele	187	212		1.93 (0.54-2.15)
T-allele	133			
TLR 2- rs893629 G/A		278	0.870	
G-allele	262	162		1.95 (0.65-2.19)
A-allele	58			
TLR 2- rs1898830 A/G		436	0.239	
A-allele	303	4		1.93 (0.58-2.98)
G-allele	17			
TLR 2- rs121917864 C/T		199	0.544	
C-allele	194	241		1.99 (0.68-2.45)
T-allele	126			
TLR 2- rs4696480 T/A		197	0.635	
T-allele	162	243		1.12 (0.76-1.45)
A-allele	158			
TLR 2- rs3804099 T/C		408	<0.0001	
T-allele	272	32		1.19 (0.87- 1.57)
C-allele	48			
TLR 2- rs5743699 T/C		436	0.681	1.01 (0.90-1.35)
T-allele	298	4		

C-allele	22			
TLR 2- rs3804100 T/C		208	0.569	1.08 (0.65-1.19)
T-allele	158	232		
C-allele	162			
TLR 4 - rs7869402 C/T		402	0.528	
C-allele	278	38		1.90 (0.60-2.35)
T-allele	42			
TLR 4 - rs1927907 G/A		430	0.536	
G-allele	300	10		1.84 (0.63- 2.12)
A-allele	20			
TLR 4 - rs1927911 C/T		196	0.255	
C-allele	162	244		1.02 (0.65- 1.61)
T-allele	158			
TLR 4 - rs1927914 T/C		396	0.225	
T-allele	274	44		1.98 (0.77-2.25)
C-allele	46			
TLR 4 - rs6478317 A/G		402	0.564	
A-allele	296	38		1.18 (1.03–1.33)
G-allele	24			
TLR 4 - rs55912718 A/G		410	0.587	
A-allele	308	30		1.06 (0.76–1.26)
G-allele	12			
TLR 4 - rs5030719 G/A		422	0.434	
G-allele	268	18		1.36 (0.72–2.55)
A-allele	52			
TLR 4 - rs10759931 C/T		218	0.737	
C-allele	193	222		1.96 (0.76–2.23)
T-allele	127			
TLR 4 - rs10759933 A/C		284	0.767	
A-allele	268	156		1.90 (0.77–2.19)
C-allele	52			
TLR 4 - rs2770150 T/C		436	0.307	
T-allele	303	4		1.12 (0.94–1.28)
C-allele	17			
TLR 4 – rs1554973 C/T				
C-allele	194	200	0.482	1.20 (0.99–2.23)
T-allele	126	240		

TLR 4 - rs11536878 C/A		196	0.881	
C-allele	184	244		1.58 (0.35–2.04)
A-allele	136			
TLR 4 - rs11536879 A/C			0.633	
A-allele	268	402		1.11 (0.95–2.25)
C-allele	52	34		
TLR 4 - rs7873784 G/C		432	0.566	
G-allele	300	8		1.14 (0.93–1.32)
C-allele	20			
TLR 4 - rs11536889 G/C		196	0.235	
G-allele	162	244		1.12 (0.60–2.07)
C-allele	158			
TLR 4 - rs4986790 A/G		406	<0.0001	
A-allele	276	34		1.31 (0.65–2.66)
G-allele	44			
TLR 4 - rs4986791 C/T			<0.0001	
C-allele	162	224		1.32 (0.88–1.187)
T-allele	158	216		
TLR 4 - rs11536897 G/A		408	0.663	
G-allele	268	32		1.40 (0.86–2.23)
A-allele	52			
TLR 4 - rs11536898 C/A		436	0.773	
C-allele	310	4		1.37 (0.83–2.26)
A-allele	10			
TLR 6- rs3796508 T/C		420	0.235	1.54 (1.05–2.16)
T-allele	302	20		
C-allele	18			
TLR 6- rs5743810 C/T		421	<0.0001	1.19 (0.85–1.76)
C-allele	275	19		
T-allele	45			
TLR 6- rs5743831 T/C		218	0.698	1.22 (0.73–2.35)
T-allele	193	222		
C-allele	127			
TLR 6- rs1039559 T/C		248	0.623	1.11 (0.96–1.50)
T-allele	164	192		
C-allele	156			

TLR 6- rs6531670 T/C		244	0.690	1.09 (0.96–1.45)
T-allele	170	196		
C-allele	150			
TLR 6- rs5743788 C/G		402	0.543	1.23 (0.53–2.43)
C-allele	276	38		
G-allele	44			
TLR 6- rs5743794 C/T		436	0.598	1.18 (0.95–2.45)
C-allele	280	4		
T-allele	40			
TLR 8- rs4830805 G/A				1.92 (0.85–4.32)
G-allele	172	218	0.530	
A-allele	148	222		
TLR 8- rs4830808 C/T				
C-allele	278	410	0.827	1.08 (0.56–2.09)
T-allele	42	30		
TLR 8- rs3747414 G/T				1.56 (0.93–2.62)
G-allele	308	436	0.517	
T-allele	12	4		
TLR 8- rs3761624 C/A				1.90 (1.40-2.56)
C-allele	301	420	1.000	
A-allele	19	20		
TLR 8- rs1548731 C/T				1.07 (0.74–1.55)
C-allele	276	424	0.629	
T-allele	44	16		
TLR 8- rs2109134 A/T				
A-allele	189	228	0.299	1.68 (1.07-2.65)
T-allele	131	212		
TLR 8- rs3788935 A/G			0.652	
A-allele	161	197		1.28 (0.74, 2.22)
G-allele	159	243		
TLR 8- rs1013150 G/A				
G-allele	274	424	0.851	1.32 (0.74, 2.38)
A-allele	46	16		
TLR 8- rs5744068 C/T			0.245	
C-allele	180	233		1.98 (1.45-2.60)
T-allele	140	207		
TLR 8- rs3764879 G/C			<0.0001	1.81 (1.31-2.44)

G-allele	200	200		
C-allele	120	240		
TLR 8- rs5744080 C/T			0.540	1.23 (0.67, 2.27)
C-allele	160	213		
T-allele	160	227		
TLR 8- rs3764880 A/G			<0.0001	1.43 (0.79-2.760)
A-allele	311	438		
G-allele	9	2		
TLR 8- rs17256081 C/T			1.000	1.10 (0.76, 1.60)
C-allele	224	430		
T-allele	96	10		
TLR 8- rs5741883 C/T				1.20 (0.92-1.57)
C-allele	158	436	0.618	
T-allele	162	4		
TLR 8- rs2407992 G/C				1.26 (0.97-1.65)
G-allele	282	380	0.543	
C-allele	38	60		
TLR 8- rs178998 G/A				
G-allele	218	416	0.833	1.15 (0.77, 1.70)
A-allele	102	24		
TLR 9- rs5743836 A/G			0.09	1.25 (0.96-1.66)
A-allele	161	197		
G-allele	159	243		
TLR 9- rs164637 G/A			0.06	0.28(0.10-0.90)
G-allele	274	404		
A-allele	46	36		
TLR 9- rs352139 T/C			0.26	1.46 (0.79-2.75)
T-allele	302	433		
C-allele	18	7		
TLR 9- rs352140 C/T			0.34	1.90 (1.40-2.56)
C-allele	314	417		
T-allele	6	23		
TLR 9- rs352143 T/C			0.20	1.20 (0.92-1.57)
T-allele	273	422		
C-allele	47	18		
TLR 9- rs352162 T/C			0.25	1.26 (0.97-1.65)
T-allele	189	228		

C-allele	131	212		
TLR 9- rs352165 A/G		282	<0.0001	
A-allele	266	158		
G -allele	54			1.91 (1.41-2.57)
TLR 9- rs352167 T/C	305	436	<0.0001	
T-allele	15	4		1.47 (0.78-2.76)
C-allele				
TLR 9- rs187084 A/G			<0.0001	
A-allele	196	197		1.98 (1.45-2.60)
G-allele	124	243		
TLR 10- rs4129009 A/G				1.36 (1.01–1.81)
A-allele	172	218	<0.0001	
G-allele	148	222		
TLR 10- rs7694115 A/G				
A-allele	278	410	0.468	1.92 (1.19–3.12)
G-allele	42	30		
TLR 10- rs10856839 A/C				1.94(1.14–3.12)
A-allele	308	436	0.729	
C-allele	12	4		
TLR 10- rs11466645 T/A				1.13 (0.84–1.45)
T-allele	301	420	0.154	
A-allele	19	20		
TLR 10- rs4274855 G/A				1.52 (0.86–2.58)
G-allele	276	424	0.370	
A-allele	44	16		
TLR 10- rs11096955 A/C				
A-allele	189	228	0.084	1.36 (0.89–2.06)
C-allele	131	212		
TLR10- rs11096956 G/T			0.446	
G-allele	161	197		1.34 (0.79–2.28)
T-allele	159	243		
TLR10- rs11096957 A/C				
A-allele	274	424	0.275	1.58 (1.01–2.47)
C-allele	46	16		
TLR 10- rs7698870 G/A			0.296	
G-allele	180	233		1.35 (0.77–2.38)

A-allele	140	207		
TLR10- rs10776483 T/C			0.543	1.47 (0.62–3.56)
T-allele	200	200		
C-allele	120	240		

P-values are calculated with the Fisher exact test.