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Identification of *HLA-DQA1* as a Susceptibility Gene for Spinal Tuberculosis by Exome Sequencing

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Data Interpretation D
Manuscript Preparation E
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Background: Spinal tuberculosis (STB) is the main cause of bone and joint tuberculosis. This study aimed to screen and analyze the susceptibility genes for STB using whole-exome sequencing (WES).

Material/Methods: All exon regions of peripheral blood DNA from 6 STB patients were captured and sequenced using WES and the sequencing data were analyzed by modern bioinformatics methods to identify disease-causing mutations. Sanger sequencing was then used to validate the mutation sites in normal controls (207) and STB patients (193). The mRNA expression of the mutant gene and the serum levels of IL-6 and TNF-α were detected using qPCR or ELISA assay, respectively.

Results: A nonsynonymous single-nucleotide polymorphism (SNP) in the gene *HLA-DQA1* (rs796778515, c.592delCinsG, CAG to GAG, p.Q198E) was identified and further validated by Sanger sequencing. The percentage of the 3 genotypes C/C, C/G and G/G in STB patients and normal controls were 37.3%, 32.1%, and 30.6% and 47.8%, 33.8%, and 18.4%, respectively. Furthermore, the C>G mutation was significantly associated with the occurrence of STB. In addition, the levels of *HLA-DQA1* mRNA were significantly lower in blood cells from STB patients compared with normal controls, while the serum levels of IL-6 and TNF-α were significantly higher.

Conclusions: The C>G mutation in the *HLA-DQA1* gene was associated with the occurrence of STB. This variation may result in the decreased level of *HLA-DQA1* mRNA and increased serum levels of IL-6 and TNF-α, which finally led the STB susceptibility.

MeSH Keywords: **High-Throughput Nucleotide Sequencing**

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Background

Tuberculosis (TB), one of the most serious threats to human health worldwide, is caused by *Mycobacterium tuberculosis* [1]. At present, TB accounts for about 15% of global tuberculosis cases and is a major public health problem in India [2]. Spinal tuberculosis (STB) is a secondary common tuberculosis, accounting for about 50% of the incidence of bone and joint tuberculosis. Several genes have been found to be associated with the onset of STB, such as *BMP-4*, *OPN,* and *MMP-1-1607* [3,4]. However, there were still few studies on the susceptibility genes for STB. In the present study, the relationship between the polymorphism of the new candidate genes and STB was explored by sequencing of the exons.

Genetic susceptibility is defined by the genetic predisposition to a disease. The concept of modern genetic susceptibility includes genetic determinants of the occurrence, the outcome, and the prognosis of the disease. Disease susceptibility is determined by a variety of factors, not just following the Mendelian genetic model. Multiple genes lead to STB genetic susceptibility, such as *NRAMP1,* the vitamin D receptor (VDR) [5–8]. Two methods are used to detect genetic susceptibility: candidate gene strategy and whole-genome scanning strategy. In recent years, exome sequencing, a method with high accuracy and exonic coverage, has been successfully used to identify pathogenic insertions or deletions (InDels) and disease-causing mutations. Ng et al. sequenced the complete genomic exons of a subject and found 10 389 nonsynonymous SNPs (nsSNPs), of which 5604 were heterozygous and 4785 were homozygous. Although most of the SNPs are common variations and are neutral, they found previously unknown genes associated with the disease [9]. Bowden et al. sequenced 3 of the 2 pedigrees with no significant difference in plasma ethanedinitrile levels using the Agilent SureSelect Exon Capture System and the Genome Analyzer IIx System, and found that the low-frequency (1.1%) mutation of the ADIPOQ gene (G45R) in 63% of the families accounts for 17% of Hispanic American plasma adiponectin levels [10]. Seshagiri et al. performed exon sequencing on 70 matched colon cancer samples (15 microsatellite instability [MSI] and 55 microsatellite-stable [MSS]) and predicted the mutation point in protein sequence and corresponding function by using SIFT, PolyPhen, and mCluster bioinformatics tools. They identified 23 mutant genes with statistical significance in MSS, in which the ATM gene plays a role in cell cycle checkpoint control, suggesting the involvement of ATM mutations in the carcinogenic process (11). In our study, we performed whole-exome sequencing (WES) in 6 STB patients to screen for the susceptibility genes for STB.

Material and Methods

Subjects and blood samples

This work was approved by the Ethics Committee of the Hangzhou Red Cross Hospital, China. All the STB patients (193, 55–68 years old) and normal control individuals (207, 56–67 years old) were recruited from the Hangzhou Red Cross Hospital, Zhejiang, China, and written informed consent was obtained from all participants. We chose the orthopedic outpatients without STB as the controls. All of the participants were of Chinese Han ethnicity, geographically located in southern China, and from unrelated families. They were clinically examined by 2 senior orthopedists for: clinical manifestations, back pain and tenderness, spine deformity and abnormal posture; bone destruction, intervertebral space narrowing or disappearance, thick deformity, and sequestrum, determined by X-ray; mild anemia, PPD, ESR increased and positive vertebral lesions and soft tissue biopsy, analyzed by biochemistry. Various biomedical markers were recorded. Peripheral blood was drawn from all subjects, centrifuged at 4°C and 2000 rpm for 10 min, and then stored at –80°C for subsequent use.

Peripheral blood DNA extraction

Genomic DNA was extracted from peripheral blood using the FlexiGene DNA kit (QIAGEN, USA) [12]. The integrity of genomic DNA (gDNA) was examined by running on a 0.9% agarose gel, and DNA quality was evaluated by Nanodrop and Qubit analysis.

Whole-exome sequencing

All exon regions of peripheral blood DNA from 6 STB patients (mean age 61±5 years) were captured and sequenced using WES. The Roche NimbleGen SeqCap EZ Human Exome Library v3.0 kit (Roche, UK) was used for exome capture according to the manufacturer's procedures. The enriched exome libraries were sequenced on an Illumina Hiseq X Ten platform (Illumina, USA) for the 150 bp reads, using the paired-end method. Low-quality reads and adapter sequences were filtered out and about 130 Mb clean reads were finally obtained for each sample.

Read mapping, variant calling, and annotation

We aligned the sequence reads in each sample to the human reference genome (GRCh37/hg19) using the Burrows-Wheeler Aligner (BWA) to get the initial comparison results [13]. We then removed the duplicated reads and mutations introduced by library construction and reserved the unique mapping reads using the Picard tools. Next, the average coverage rate and the target depth were analyzed. The single-nucleotide variants (SNVs) and the insertions or deletions (InDels) were identified using the Unified Genotyper module in the GATK software. The identified

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SNPs and InDels were then annotated using ANNOVAR software. After being filtered out based on multiple databases, including the Consensus CDS (CCDS) database, the dbSNP database V147, and the human genome builder NCBI 37, the common SNPs were removed from the data and compared with the normal exon database from Shanghai Xiang Yin Company. Only SNVs with a Phred-scaled SNV quality ≥20, a read coverage ≥4×, and a distance between 2 adjacent SNVs ≥5 bp were reserved [14]. We finally identified 13 related genes (data not shown).

PCR and Sanger sequencing

The mutation sites were further validated by Sanger sequencing after PCR amplification in normal controls (207) and STB patients (193). PCR was performed using a 10×PCR buffer (Takara, China), 10 ng of gDNA template (the peripheral blood DNA), 0.5 mM of each primer, and Polymerase Takara *EX* Taq (Takara, China). The reactions conditions were as follows: pre-denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 40 s, annealing at 60/65°C for 50 s, and extension at 72°C for 1 min. The Sanger sequencing was performed according to the manufacturer's specifications using the bi-directional PCR primers, and then the genotypes were determined.

Detection of the expression levels of HLA-DQA1 by qPCR

We performed qPCR (quantitative real-time PCR) to detect the expression levels of *HLA-DQA1* from 30 normal people and 30 STB patients. Briefly, total RNA was extracted from the blood samples using TRIzol reagent (Invitrogen, Life Technologies), and were treated with DNase I (Fermentas, Thermo). Then, the first-strand cDNA was synthesized using a ReverTra Ace-a Kit (Toyobo), and real-time PCR was performed with a QuantiFast SYBR Green PCR Kit (Qiagen). The qPCR conditions were as follows: 95°C, 10 min; (95°C, 15 s; 60°C, 45 s)×40 cycles. Quantification was performed by using the comparative Ct method ($2^{-\Delta\Delta Ct}$). The primers used for qPCR were:
HLA-DQA1-F: AGGCTTAGGGGAAGAGTGA;
HLA-DQA1-R: CTTGTCTGTCAAGTTCAGAA;
β-actin-F: CATCGTCCACCGCAAATGCTTC;
β-actin-R: AACCGACTGCTGTCACCTTCAC.

Measurement of IL-6 and TNF-α in the serum by ELISA assays

We measured the serum levels of IL-6 and TNF-α using ELISA kits (Abcam, #ab178013 and Abcam, #ab181421) in 30 normal people and 30 STB patients according to the manufacturer's instructions.

Common biochemical assays

We performed common biochemical assays on all serum samples from 207 normal people and 193 STB patients. The levels of total cholesterol (TC), C-reactive protein (CRP), lipoprotein (a) (Lp (a)), albumin (Alb), erythrocyte sedimentation rate (ESR), low-density lipoprotein (LDL), and eosinophils were examined by routine biochemical examination methods.

Statistical analysis

Statistical analyses were carried out using SPSS 16.0 (IBM, Armonk, NY). Quantitative data in accordance with the normal distribution of data are presented as mean ± standard deviation. The independent-samples *t* test or Pearson chi-square (χ^2) test was used to analyze the differences between 2 groups, for the quantitative data or qualitative data, respectively. The genotypic and allelic frequencies were evaluated using logistic regression analyses by computing the odds ratio (OR) and 95% confidence intervals (95% CIs). *P* values less than 0.05 were considered as significantly different.

Results

Identification of susceptibility genes by exome sequencing

To identify susceptibility genes in STB patients, we performed WES analysis on the peripheral blood DNA samples from 6 patients with STB. We first confirmed that the exome sequencing data has passed sequencing quality standards with the average coverage rate for 50Mb exome higher than 200× and the target depth for 99% of the nucleotides higher than 20×, indicating that these data could be used for further analysis (Table 1).

We then applied a series of data filtering methods, as mentioned in Methods, to identify disease-causing mutations in STB, and finally obtained 13 susceptibility genes. Among these genes, the *HLA-DQA1* gene was especially noteworthy because it was reported to be involved in anti-tuberculosis immune responses [15]. For the gene *HLA-DQA1*, 1 synonymous SNP (C>T at the position 591) and 1 nonsynonymous SNP (C>G at the position 592) were identified. The nonsynonymous SNP caused the codon shift from CAG to GAG and the amino acid shift from Q (glutamine) to E (glutamic acid) at position 198 (Figure 1, Table 2).

We then validated the 2 SNPs by Sanger sequencing in normal controls (207) and STB patients (193). We found that the amino acid at the position 198 of HLA-DQA1 was conservative (either Q or E) in different species (Figure 1B).

Relationship between HLA-DQA1 Polymorphism and STB

In STB patients, there were 3 genotypes – C/C, C/G, and G/G – with the percentage of 37.3%, 32.1%, and 30.6%, respectively, while the normal individuals had a different distribution

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Table 1. Overview of the exome sequencing data.

Sample	16R01088	16R01089	16R01090	16R01091	16R01092	16R01093
Clean reads	162.54	168.51	156.84	168.16	153.9	139.97
Average length	144	144	144	143	143	144
Average base quality	40.2	40.3	40.3	40.3	40.3	40.2
Average lab size	210.1	202.7	201.5	198.4	206.5	219.7
Align rate (%)	99.22	99.11	99.14	99.06	99.12	99.3
Total target base	50390601	50390601	50390601	50390601	50390601	50390601
Covered target base	50325031	50327581	50378974	50378430	50326719	50378077
Coverage rate (%)	99.87	99.87	99.98	99.98	99.87	99.98
Total effective base (Mb)	16923.17	17556.87	16741.6	17657.22	16267.82	15331.93
Effective base on target (Mb)	11041.87	11572.39	11123.45	11746.58	10765.87	9944.34
Capture rate (%)	65.25	65.91	66.44	66.53	66.18	64.86
Target average depth	219.13	229.65	220.74	233.11	213.65	197.35
Target 4× rate (%)	99.82	99.82	99.94	99.94	99.82	99.93
Target 10× rate (%)	99.68	99.71	99.81	99.83	99.68	99.76
Target 20× rate (%)	99.28	99.38	99.43	99.49	99.25	99.28

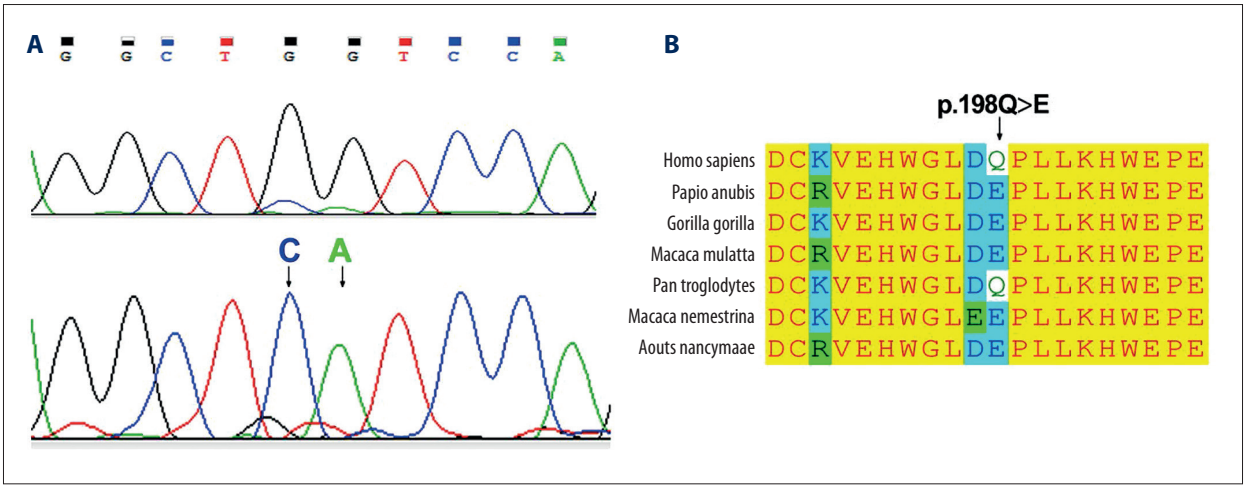

Figure 1. Nucleotide change and amino acid change of single-nucleotide polymorphisms (SNPs) identified by exon sequencing. (**A**) The representative Sanger sequencing chromatograms of the identified mutations. The mutation positions are indicated by arrows. The bases containing the corresponding mutation sites from the reverse complementary strand are shown in the chromatogram because the sequencing in these 2 regions was performed on the antisense strand. The C>T mutation at the position 591 was a synonymous SNP and the C>G mutation at the position 592 was a nonsynonymous SNP. (**B**) Comparative protein alignment of HLA-DQA1 protein in *Homo sapiens*, *Papio anubis*, *Macaca mulatta*, *Gorilla gorilla*, Macaca mulatta, *Pantroglodytes* and *Aotus nancymaae*. The mutated amino acid is indicated by the arrow.

Table 2. Brief information of SNP rs796778515.

Gene symbol	Gene ID	Mutation type	Chr. (position)	Nucleotide change	Protein level change
HLA-DQA1	NM_002122	SNV	chr6.32610008 -32610009	c.591_592 delCCinsTG	p.Q198E

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Table 3. The distribution of genotypes and chi-square (χ^2) test.

HLA-DQA1 (C>G)	n	Genotypes (%)			Pearson chi-square (χ^2)	*P* value
		C/C	C/G	G/G		
Normal	207	99 (47.8)	70 (33.8)	38 (18.4)	8.815	0.012
Patients	193	72 (37.3)	62 (32.1)	59 (30.6)		

Table 4. Genotype and allele analysis.

Genotype and minor allele	Patients (%)	Normal (%)	P value for model of inheritance and OR (95% CI)		
			Additive model	Dominant model	Recessive model
G/G	59 (30.6)	38 (18.4)	0.003, 0.468 (0.282, 0.779)	0.034, 1.541 (1.033, 2.297)	0.003, 0.511 (0.320, 0.814)
C/G	62 (32.1)	70 (33.8)	0.398, 0.821 (0.520, 1.297)		
C/C	72 (37.3)	99 (47.8)	1		
G	180 (46.7)	146 (35.3)	0.019, 0.708 (0.531, 0.944)		

with the percentage of C/C, C/G, and G/G at 47.8%, 33.8%, and 18.4%, respectively. Furthermore, the G/G genotype was in 30.6% and 18.4% of STB patients and normal controls, respectively, indicating that the prevalence of the variations is significantly higher in STB patients compared with controls (P<0.05) (Table 3). The χ^2 test result demonstrated that there was a significant difference in the distribution of 3 genotypes (C/C, C/G, G/G) of the gene *HLA-DQA1* between the patient group and normal group (*P*=0.012) (Table 3), preliminarily indicating that the C>G mutation of the gene *HLA-DQA1* is associated with the occurrence of STB.

We then explored the correlation of the genotype and STB by genotype and allele analysis and found that the genotype G/G in the gene *HLA-DQA1* was significantly associated with the occurrence of STB in the additive model, the dominant model, and the recessive model (*P*=0.003, 0.034, and 0.003, respectively). In contrast, the genotypes C/G and C/C in the gene *HLA-DQA1* had no correlation with the occurrence of STB in the models tested (*P*>0.05), and the minimum allele G was significantly associated with the incidence of STB (P<0.05) (Table 4). These results suggest that the HLA-DQA1 polymorphism is a major contributor to STB.

The HLA-DQA1 expression levels in serum

To test whether the missense variants (C/C>G/G) affect the gene expression of HLA-DQA1, we performed qPCR analysis. We found that the expression levels of HLA-DQA1 were significantly lower in STB patients compared with normal controls (*P*<0.05) (Figure 2), suggesting that these missense variants

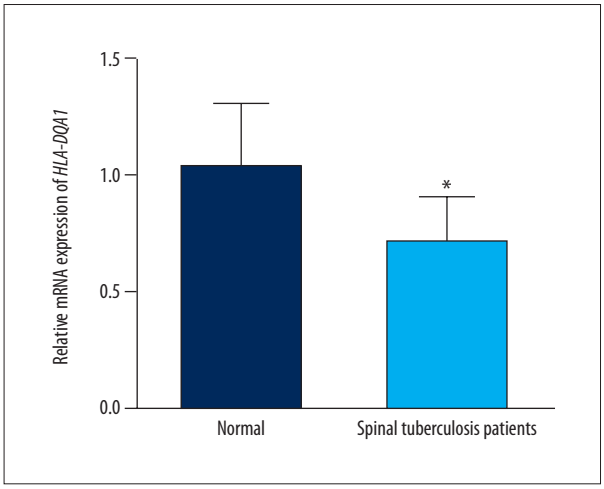

Figure 2. The expression of the gene *HLA-DQA1* mRNA in the normal (n=30) and spinal tuberculosis patients (n=30). * *P*<0.05, significant difference.

in *HLA-DQA1* decreased its expression. These results are consistent with the role of *HLA-DQA1* in anti-tuberculosis immune response.

The levels of chemokine, inflammatory factors, and other biochemical factors in serum

Next, we examined the levels of IL-6 and TNF-α, which are 2 primordial regulators in tuberculosis [16]. The results showed that the levels of IL-6 and TNF-α were significantly higher in the STB patients than those in normal people (Table 5).

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Table 5. Comparison of the serum levels of IL-6 and TNF-α in the normal and STB patients.

Group	IL-6 (pg/mL)	TNF-α (pg/mL)
Normal (n=30)	80.81±14.95	44.30±9.08
Patients (n=30)	126.32±15.98*	70.49±11.93*

* *P*<0.05, significant difference.

Table 6. Comparison of the biochemical indexes between the normal and STB patients.

	Normal (n=207)	Patients (n=193)	*P* value
TC (mmol/L)	4.19±1.45	4.27±2.33	0.408
Lp (A) (mg/dL)	25.34±0.22	155.83±0.35	0.018
CRP (mg/L)	3.07±1.21	24.67±1.66	0.008
Alb (g/L)	41.52±1.66	35.5±1.81	0.005
ESR (mm/1 h)	17.45±1.20	85.0±1.41	0.021
Eosinophils (10^9/L)	0.27±0.51	0.4±0.45	0.002
LDL (mmol/L)	1.87±0.75	1.96±0.82	0.562

Data are expressed as mean ±SD. *P* values were obtained from independent-samples *t* test (TB *vs.* control). TC – total cholesterol; CRP – C-reactive protein; Lp(A) – lipoprotein(a); Alb – albumin; ESR – erythrocyte sedimentation rate; LDL – low-density lipoprotein.

Consistent with this, we also assessed the levels of other chemokines, inflammatory factors, and biochemical factors by routine biochemical examination method. We found that the levels of Lp (a), CRP, ESR, and eosinophils were significantly higher in the STB patients than those in normal people. In contrast, the Alb levels were significantly lower in STB patients compared with normal controls. However, no difference was found in the levels of TC and LDL between the 2 groups (Table 6).

Discussions

The spine is the supporting trunk of the human body that protects the internal organs and spinal cord. STB is a form of tuberculosis with high disability rate. As reported, extrapulmonary tuberculosis and bone tuberculosis account for 15–20% and 10% of all tuberculosis cases, respectively [17], and TBS accounts for 50% of bone tuberculosis cases [17]. It has been reported that up to 60% of bone tuberculosis is involved in HIV co-infection, and is more common in immunosuppressed individuals [18]. The World Tuberculosis Report of the World Health Organization (WHO) in 2015 estimated that there were 480 000 cases of multi-drug-resistant tuberculosis (MDR-TB) worldwide in 2014 and 15 000 of these cases were pulmonary tuberculosis in the eastern Mediterranean region [19]. There are probably 5000 cases of MDR-STB worldwide and about 150 cases of them per year were in the eastern Mediterranean region. Most of the cases came from countries with high TB burdens like India, China, and South Africa. In the United States, during the 2006–2011 survey, the incidence of STB was found to decrease significantly over time, reaching 1 case per 2 million people in 2011. However, only about 20% of patients with STB underwent surgery. Therefore, STB remains a public health concern and usually affects middle-aged men [20]. From twins and case-control studies, Bellamy et al. found that the host gene plays an important role in the pathogenesis of STB [21], and the environment and the host immunity also have a great influence on STB. So, in order to further explore the pathogenesis of STB and improve the condition of STB patients, more susceptibility genes need to be screened.

Some susceptibility genes of tuberculosis have been extensively studied, such as *MCP-1-2518* locus gene, *HLA* gene, and *Interleukin-12* gene [22–24]. Most of these genes that encode the proteins of the tuberculosis immune response are closely related to the proteins involved in the anti-tuberculosis immune response in all aspects.

The human leukocyte antigen (HLA)-DQ molecule is a glycoprotein heterodimer composed of 1 α-chain and 1 β-chain, encoded by the *HLA-DQA* locus and *HLA-DQB* locus, respectively. These antigens are expressed on the surface of antigen-presenting cells and play a key role in the immune recognition of foreign and self-produced antigens [25,26]. The polymorphisms defining the alleles of this HLA class II gene are located in a 242-bp region (or 239 bp for alleles 2 and 4) within the second exon of the *HLA-DQA* gene. Eight alleles have been identified and designated as DQA1*0101, *0102, *0103, *0201, *0301,

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*0401, *0501 and *0601 [26]. Many studies have shown that expression of HLA-DQA1 is associated with the occurrence of certain diseases. Kim et al. performed PCR-SSOP to do DQA1 genotyping in 18 Korean Vogt-Koyanagi-Harada (VKH) patients and 128 healthy controls and found that DQA1*0302 was associated with VKH and DQA1*0101, *0102, *0103, and *0501 were associated with resistance [27]. A study using a mouse model found that the susceptibility to glomerular basement membrane disease is associated with the *AβAα* region, corresponding to the human *HLA-DQ* region, and thus supporting the importance of the *HLA-DQA1* allele in immune-related glomerular diseases [28,29]. As reported, HLA-DQA1 has been found to be associated with various immune-related diseases, but not with STB. In the present study, through gene sequencing and comparison of the case group and normal control group, we confirmed the polymorphism of *HLA-DQA1* gene and its association with the occurrence of STB. In addition, this mutation resulted in decreased levels of *HLA-DQA1* mRNA. These results further support the hypothesis that *HLA-DQA1* is a susceptibility gene for STB and provide an experimental basis for the clinical treatment and drug development of STB.

The consequences of the C>G mutation of the gene *HLA-DQA1* are as follow: (1) the amino acid change is Q>E; (2) this variation did not change the total number of amino acid residues of the HLA-DQA1 protein; (3) the pI of Q and E is ~5.6 and ~3.2, respectively; (4) the SNPs are located in the exon 3 of *HLA-HQA1*, encoding the peptide outside of the membrane; (4) as the *in vivo* pH is around 6.8–7.2 in humans, the amino acid change from Q to E would cause the peptide to be more negatively charged, leading to the conformation changes of the protein, especially for the peptide outside. Our results clearly show the polymorphism and susceptibility of *HLA-DQA1* in STB.

IL-6 and TNF-α are 2 primordial regulators of tuberculosis (16). Previous studies have shown that the host immunity is highly correlated with the development of STB [18,21]. In the present study, the IL-6 and TNF-α levels were both significantly higher in the STB patients than those in normal controls; the Lp (a), CRP, ESR and eosinophils levels were significantly higher in the STB patients compared with normal patients; and the Alb levels were significantly lower in ST patients than those in normal controls. These results show that the immunity of STB patients was low, the patients were susceptible to infection, and the inflammatory factors were higher in the STB patients than those in normal controls, which is consistent with previous studies. In addition, these results suggest that using the combination of HLA-DQA1 level, IL-6 level, TNF-α level, and common biochemical assays might facilitate the early diagnosis of STB.

Conclusions

In summary, in the Chinese Han population, the polymorphism of *HLA-DQA1* gene (rs796778515, c.592delCinsG) is associated with the occurrence of STB. This mutation resulted in the decreased transcriptional levels of *HLA-DQA1* and increased levels of serum inflammatory factors IL-6 and TNF-α, which might finally lead to STB susceptibility. The identification of *HLA-DQA*1 as a susceptibility gene for STB provides an experimental basis for the early diagnosis, clinical treatment, and drug development of STB.

Conflicts of interest

None.

References:

1. Cormican L, Hammal R, Messenger J, Milburn HJ: Current difficulties in the diagnosis and management of spinal tuberculosis. Postgrad Med J, 2006; 82(963): 46–51
2. Glaziou P, Floyd K, Raviglione M: Global burden and epidemiology of tuberculosis. Clin Chest Med, 2009; 30(4): 621–36
3. Wang G, Xie L, Hu J et al: Osteopontin, bone morphogenetic protein-4, and vitamin D receptor gene polymorphisms in the susceptibility and clinical severity of spinal tuberculosis. Cell Physiol Biochem, 2017; 41(5): 1881–93
4. Zhou Y, Gao Q, He D et al: Matrix metalloproteinase-1 promoter -1607 bp 1G/2G polymorphism associated with increased risk of spinal tuberculosis in Southern Chinese Han population. J Clin Lab Anal, 2017; 31(6): 22136
5. Tiksnadi B, Herman H: The NRAMP1 polymorphism as a risk factor for tuberculous spondylitis. Malays Orthop J, 2013; 7(1): 25–29
6. Li X, Yang Y, Zhou F et al: SLC11A1 (NRAMP1) polymorphisms and tuberculosis susceptibility: Updated systematic review and meta-analysis. PLoS One, 2011; 6(1): e15831
7. Zhang H-Q, Deng A, Guo C-F et al: Association between FokI polymorphism in vitamin D receptor gene and susceptibility to spinal tuberculosis in Chinese Han population. Arch Med Res, 2010; 41(1): 46–49
8. Selvaraj P, Kurian S, Chandra G et al: Vitamin D receptor gene variants of BsmI, ApaI, TaqI, and FokI polymorphisms in spinal tuberculosis. Clin Genet, 2004; 65(1): 73–76
9. Ng PC, Levy S, Huang J et al: Genetic variation in an individual human exome. PLoS Genet, 2008; 4(8): e1000160
10. Bowden DW, An SS, Palmer ND et al: Molecular basis of a linkage peak: Exome sequencing and family-based analysis identify a rare genetic variant in the ADIPOQ gene in the IRAS Family Study. Hum Mol Genet, 2010; 19(20): 4112–20
11. Seshagiri S, Stawiski EW, Durinck S et al: Recurrent R-spondin fusions in colon cancer. Nature, 2012; 488(7413): 660–64
12. Dauber A, Stoler J, Hechter E et al: Whole exome sequencing reveals a novel mutation in CUL7 in a patient with an undiagnosed growth disorder. J Pediatr, 2013; 162(1): 202–4.e1
13. Li H, Durbin R: Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics, 2009; 25(14): 1754–60
14. Wang K, Li M, Hakonarson H: ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res, 2010; 38(16): e164
15. Goldfeld AE, Delgado JC, Thim S et al: Association of an HLA-DQ allele with clinical tuberculosis. JAMA, 1998; 279(3): 226–28
16. Mattos AMM, Almeida CdS, Franken KL et al: Increased IgG1, IFN-γ, TNF-α and IL-6 responses to *Mycobacterium tuberculosis* antigens in patients with tuberculosis are lower after chemotherapy. Int Immunol, 2010; 22(9): 775–82

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17. Polley P, Dunn R: Noncontiguous spinal tuberculosis: incidence and management. Eur Spine J, 2009; 18(8): 1096–101
18. Moon MS: Tuberculosis of the spine. Controversies and a new challenge. Spine, 1997; 22(15): 1791–97
19. World Health Organization: Global tuberculosis report 2015. Geneva: World Health Organization, 2015
20. De la Garza Ramos R, Goodwin CR, Abu-Bonsrah N et al: The epidemiology of spinal tuberculosis in the United States: An analysis of 2002–2011 data. J Neurosurg Spine, 2017; 26(4): 507–12
21. Bellamy R: Interferon-gamma and host susceptibility to tuberculosis. Am J Respir Crit Care Med, 2003; 167(7): 946–47
22. Gao Q, Du Q, Zhang H et al: Monocyte chemotactic protein-1–2518 gene polymorphism and susceptibility to spinal tuberculosis. Arch Med Res, 2014; 45(2): 183–87
23. Roth DE, Soto G, Arenas F et al: Association between vitamin D receptor gene polymorphisms and response to treatment of pulmonary tuberculosis. J Infect Dis, 2004; 190(5): 920–27
24. Tso HW, Lau YL, Tam CM et al: Associations between IL12B polymorphisms and tuberculosis in the Hong Kong Chinese population. J Infect Dis, 2004; 190(5): 913–19
25. Stern LJ, Brown JH, Jardetzky TS et al: Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. Nature, 1994; 368(6468): 215–21
26. Abba MC, Gomez MA, Golijow CD: HLA-DQA1 allele typing by nonisotopic PCR-LIS-SSCP. Braz J Med Biol Res, 2001; 34(7): 867–69
27. Kim MH, Seong MC, Kwak NH et al: Association of HLA with Vogt-Koyanagi-Harada syndrome in Koreans. Am J Ophthalmol, 2000; 129(2): 173–77
28. Kalluri R, Danoff TM, Okada H, Neilson EG: Susceptibility to anti-glomerular basement membrane disease and Goodpasture syndrome is linked to MHC class II genes and the emergence of T cell-mediated immunity in mice. J Clin Invest, 1997; 100(9): 2263–75
29. Jin Y, Birlea SA, Fain PR et al: Variant of TYR and autoimmunity susceptibility *loci* in generalized vitiligo. N Engl J Med, 2010; 362(18): 1686–97

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