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The association of single-nucleotide polymorphisms of the SP110 gene with susceptibility to latent tuberculosis infection in the Iraqi

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Abstract---Background: SP110 plays an important role in microbial immunity and regulating macrophage biological activity, which may protect against tuberculosis. To investigate whether SNPs of SP110 are associated with susceptibility to latent TB infection in an Iraqi population. Hence, identifying SNPs in these genes could be used as a marker to screen individuals at risk of latent TB infection. Method: A case-control study was conducted to investigate the polymorphism of the SP110 gene by the Sanger sequencing method in 50 individuals with latent TB infection and 40 healthy controls. Result: The findings demonstrated that the A allele and the genotype "GA" of rs9061 was significantly associated with LTBI risk ($p=0.033$; OR: 2.32; 95 %CI: 1.07 - 5.03 and $p=0.035$; OR: 3.14; 95 % CI: 1.08 - 9.07), respectively. In addition, the results of the current study showed that the genotype CT of rs28930679 was significantly associated with the risk of latent TB infection ($p=0.046$; OR:2.64;95%CI:1.02 - 6.86). Conclusion: The results found that genetic variants of the SP110 (rs9061, rs28930679) are associated with susceptibility to latent TB and single nucleotide polymorphism of the SP110 gene show the predictability of latent TB risk and subsequent TB development. And therefore , rs9061 may be a predictive genetic marker for latent tuberculosis.

Keywords---SP110, polymorphism, latent TB infection, sanger sequencing.

Introduction

It is estimated that one-fourth of the human population has been exposed to *Mycobacterium tuberculosis* (Mtb) and carries the infection in its latent form. Latent tuberculosis infection (LTBI) is a persistent immune response to *Mycobacterium tuberculosis* antigen stimulation that occurs in the absence of clinically manifested active tuberculosis (TB) disease. TST or interferon-release assays (IGRA) can be used to diagnose latent tuberculosis (LTBI) (Chang *et al.*,2018). People with latent tuberculosis infection serve as a reservoir for active TB cases. Detection and management of latent tuberculosis infection (LTBI) are now essential to the World Health Organization's End TB initiative. This is due to the fact that people who have LTBI can develop active tuberculosis or undergo reactivation, and the risk is significantly higher when predisposing factors are present (Kiazyk & Ball,2017). Variations in the genes of the host could have a significant impact on the pathogenesis of tuberculosis in humans.

Host genetic polymorphisms have been shown to influence the outcome of Mtb infection. It has been demonstrated that the SP110 gene, which encodes an interferon-induced nuclear protein, regulates innate host immunity to Mtb infection. This gene, found on chromosome 2q37.1, encodes the SP110 nuclear body protein, which is a component of cellular structures (Wu *et al.*,2015). SP110 protein may regulate macrophage biological activity and influence *M. tuberculosis* growth and proliferation by participating in signal transmission processes between nuclear hormone receptors, which may be associated with susceptibility to TB (Zhou and Li, 2006; Cai *et al.*,2013). Detailed investigation revealed that Sp110 regulates genes involved in immune responses, apoptosis, defence responses, and inflammatory responses, according to transcriptome analysis. Sp110 regulates cytokines, chemokines, and genes that regulate Mtb intracellular survival. Moreover, Sp110 regulates macrophage miRNA expression (Wu *et al.*, 2015). IFNs regulate SP110 gene expression, suggesting that its function is related to IFN-mediated immunity (Kadereit *et al.*,1993). SP110 is highly expressed in human peripheral blood leukocytes and the spleen and less so in the lung (Bloch *et al.*,2000). In humans, SP110 variants have been implicated in hepatic veno-occlusive disease with immunodeficiency (VODI)(Cliffe *et al.*,2012), viral hepatitis infection (Saito *et al.*,2004), and TB (Png *et al.*,2012), indicating the gene plays an essential role in immunity. The study aimed to investigate whether SNPs of SP110 are associated with susceptibility to LTBI TB infection in an Iraq population. Identifying SNPs in this gene could be used as a marker to screen individuals at risk of latent TB Infection and subsequent TB progression.

Materials and Methods

Ethical approval and consent

All subjects involved in this work were informed, and the agreement was obtained verbally from each one before collecting samples. The committee approved this study of publication ethics at the college of medicine, Babylon university, Iraq.

Study design and settings

The present study was designed as a case-control study, and this study was performed from February 2021 – to September 2021 at the National Tuberculosis Institute (NTI) / National Reference Laboratory (NRL) for Tuberculosis in Baghdad. All participants were divided into two groups: 50 subjects who were QFT-Plus positive at recruitment were defined as latently TB infected (LTBI). The diagnosis of Latent TB infection was based on a positive QFT-Plus test and the absence of any clinical, microbiological, or radiological features that would suggest active disease.. A 40 healthy control individual was asymptomatic with a normal chest x-ray, and a QFT- Plus negative result was included in this study. Healthy controls (HC) were defined as individuals with no TB infection or disease. The results of the QuantiFERON-TB Gold In-Tube assay (QFT) (Qiagen, Germany) were interpreted in accordance with the criteria specified by the manufacturer. The exclusion criteria included patients with autoimmune diseases, diabetes mellitus, and nephropathy. Subjects had close contact for TB with an indeterminate QFT result or a QFT negative. Also, the subjects who refused to participate in this study.

DNA extraction and genotyping

Five ml of freshly venous blood were collected from each participant at the time of diagnosis. The blood was kept in an EDTA tube and later subjected to extraction of DNA . The ReliPrep™ Blood gDNA Miniprep system was used to extract human genomic DNA (gDNA) from whole blood samples of Latent TB infection and control groups. The procedure was carried out in accordance with the method recommended by the manufacturer (Promega/USA). The extracted blood genomic DNA was examined using a Nanodrop spectrophotometer (Thermo Fisher Scientific/USA), which measured DNA concentration (ng/L) and DNA purity by reading absorbance at (260 /280 nm). The quality of the DNA was determined using 1 % agarose gel electrophoresis. The extracted DNA was kept at -20 degrees Celsius for future SNP analysis.

Primers of PCR used in DNA amplification

In the present study, two primers were designed to detect the SNPs of SP110 genes under interest by Sanger sequencing: The primer pair of targeted regions of the Sp110 gene, including SNPs: rs9061, rs11556887 respectively, the primers detail information as shown in table 1.

Gene	SNPs	Primer sequence 5' to 3'	Amplicon (bp)
Sp110	rs9061; rs11556887	F-ACGCATGTTCTCCCTTCACA	804

	R-CCCCTTCCCCAAGTAATTCTGA	
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F= forward, R= reverse, bp = base pair

SNP genotyping and selection

The candidate SNPs for this study were chosen based on a review of previous studies' literature and in silico functional prediction from the National Center for Biotechnology Information (NCBI) website (<https://www.ncbi.nlm.nih.gov/snp>). Also, SNPs were selected if they had been linked to disease and were predicted to affect function. SNPs were genotyped using the Sanger method of DNA sequencing.

Analysis of Sanger sequencing data

The sequencing results of various PCR products were edited, aligned, and analyzed with the respective sequences in the reference database using a generous prime purchased version reading program. Furthermore, the NCBI data tools were used to align the gene sequence using the NCBI BLAST tool. Each sequenced sample's observed variations were numbered in PCR amplicons and their corresponding position within the referring genome.

Statistical analysis

The statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010 were used to analyze and present the data. Genotypes were presented as percentage frequencies. Allele frequencies were determined by the direct count method. Comparison between latent TB infection and healthy controls of the distribution of SP110 SNPs was performed by Chi-square tests or Fisher's exact test. The odds ratio and 95% confidence interval were estimated to measure risk. The online calculator was used to determine if two alleles deviated significantly from the Hardy-Weinberg equilibrium. (<https://www.had2know.org/academics/hardy-weinberg-equilibrium-calculator-2-alleles.html>). *P* values < 0.05 were considered significant.

Results

Conventional polymerase chain reaction (PCR)

The results of conventional PCR showed success the primer pair efficiency to amplification target DNA region of SP110 included two SNPs included :SNPs rs9061 G>A, rs11556887 C> T, the amplification region with flanking primers and PCR product size is 804bp as indicated in the figure (1).

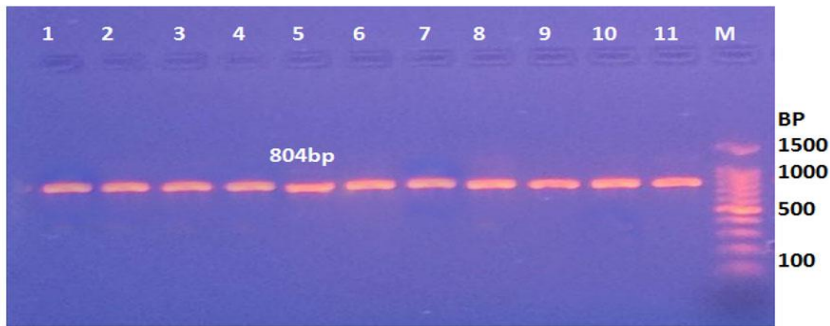
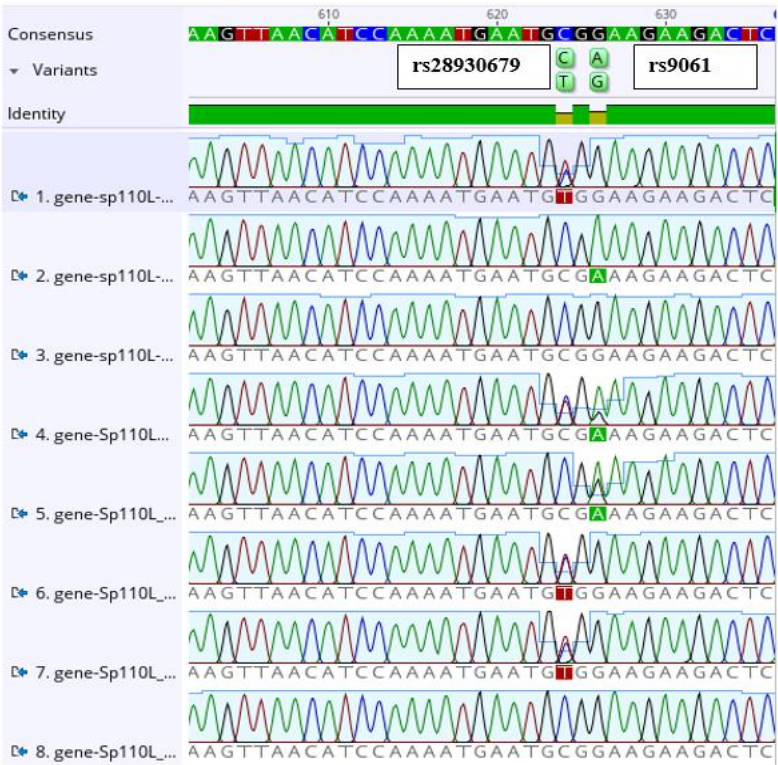


Figure (1): Image of an agarose gel electrophoresis displaying the PCR products obtained from amplifying of partial sequence of Sp110 gene included SNPs: rs9061 G>A, PCR product size is 804 bp, M=molecular marker 100bp for the first step, Lane:1 -6 for Latent TB infection (LTBI) group, Lane:7 -11 for healthy control, electrophoresis was done on 1% agarose, TBE buffer (1X),100 V for 45 min.

DNA sequencing of partial sequence of the SP110 gene revealed rs11556887 that were not polymorphic were not further included in the analysis, and the remaining 2 SNPs (rs9061 G>A and rs28930679 C> T) were then analyzed. DNA sequencing revealed rs9061 G>A and rs28930679 C> T with three genotypes GG, GA, AA and CC, CT, and TT, respectively, as shown in figure (2).



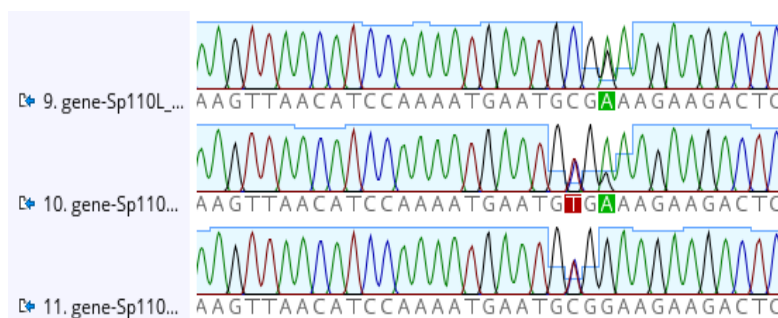


Figure (3-24): Sanger sequencing result. Multiple alignments of partial Sp110 sequences of latent TB group, based on Chromatograms peaks. Two SNP were observed: rs9061 G>A, rs28930679 C>T, alignment performed by Geneious prime software.

The allele and genotype frequencies of the two SP110 SNPs included (rs9061, rs28930679) in the LTBI cases and healthy control are shown in Tables (1). Both populations agreed with HWE for all polymorphisms tested ($P > 0.05$). The results demonstrated that the A allele and the genotype "GA" of rs9061 was significantly associated with LTBI risk ($P=0.033$; OR: 2.32;95%CI: 1.07 - 5.03 and $P=0.035$; OR: 3.14; 95% CI: 1.08 -9.07), respectively as shown in Table (1).In addition, the results of the current study showed that the genotype CT of rs28930679 was significantly associated with LTBI risk ($P=0.046$; OR:2.64;95%CI:1.02 - 6.86), as shown in table (1).

Table (1): Allele and Genotype frequencies of SP110 SNPs (Rs9061 G>A, rs28930679 C>T) in LTTB vs. healthycontrol groups

SNP ID	Location	Genotype	Latent TB N(%)	Control N(%)	OR (95%CI)	P-value
Rs9061	Exon5	GG	28 (56%)	31 (77.5%)	Reference	
		GA	17(34%)	6 (15%)	3.14 (1.08 -9.07)	0.035
		AA	5 (10%)	3 (7.5%)	1.85 (0.40 -8.44)	0.430
		HWE- <i>p</i>	0.33	0.097		
Allele frequency		G	73 (73%)	69 (86.25%)	Reference	
		A	27(27%)	11 (13.75%)	2.32(1.07 - 5.03)	0.033
rs28930679	Exon5	CC	20 (40 %)	24(60%)	Reference	
		CT	22 (44 %)	10 (25%)	2.64 (1.02 - 6.86)	0.046
		TT	8 (16 %)	6 (15 %)	1.60 (0.40 to 6.57)	0.448
		HWE- <i>p</i>	0.63	0.20		
Allele frequency		C	62 (62%)	54 (67.5%)	Reference	
		T	38 (38%)	26 (32.5%)	1.27(0.69 -2.36)	0.444

OR: odds ratio; CI: confidence interval; S: significant at $P < 0.05$. Values in bold face are statistically significant. HWE: Hardy-Weinberg equilibrium .

Discussion

There is growing evidence that the SP110 polymorphism plays a role in tuberculosis. The genetic studies suggest that the SP110 gene is essential in

modulating susceptibility to latent and active tuberculosis infection. SP110b protein acts as a host immunity proinflammatory cytokines regulator, thereby reducing tissue damage caused by excessive inflammation (Leu *et al.*, 2017). Despite the fact that many groups have studied the gene's association with TB susceptibility in various populations, the results have been inconclusive.

The results of the current study indicated that genetic markers rs9061 and rs28930679 in SP110 were linked to latent TB risk. These results were partially compatible with a study conducted in China where found that the genetic variant rs9061 in the SP110 gene may increase the risk of TB (Zhang *et al.*, 2017). In the West African population, Tosh and colleagues reported that SNPs in the SP110 gene were linked to TB (Tosh *et al.* 2006). Other studies identified the association of SP110 polymorphisms with TB by a case-control design (Cai *et al.*, 2013; Cong *et al.*, 2010). In contrast, some studies reported no significant association between rs9061 and TB risk in Asians (Lei *et al.*, 2012), Indonesians (Png *et al.*, 2012), Vietnamese (Fox *et al.*, 2014) and Han Chinese population (Jiang *et al.* 2016). Ethnic differences may contribute to the discrepancies between the previous results and the current data.

Deng *et al.* (2017) demonstrated that Exonic Single nucleotide polymorphism has a direct effect on protein properties. The SNP rs9061(G>A) is found in the exon region. The substitution of guanine for adenine at codon position 207 of the SP110 protein results in a change in amino acid from glutamic acid to lysine. The conversion of an acidic amino acid to an essential amino acid may result in a change in the protein structure or posttranslational modification of the SP110 protein. It has been proposed that the A allele may cause differences in the secondary structure of the SP110 protein's alpha helices and beta-sheets compared to the G allele (Chang *et al.*, 2018). Thus, a change in amino acid affects the function of the SP110 protein, which in turn affects the development of tuberculosis (Bellamy, 2005). More functional studies are needed to fully comprehend the implications of the single nucleotide polymorphism (rs9061G>A) on the SP110 gene and illness risk.

The genetic variants in the SP110 (rs9061 G>A, rs28930679 C> T) demonstrated the ability to predict the risk of latent TB infection and may be associated with subsequent TB progression. Global TB eradication efforts have concentrated on detecting and treating LTBI cases to control and eradicate TB. Therefore, rs9061 G>A and rs28930679 C> T SNPs in SP110 were shown in this study to be linked with LTBI susceptibility and may thus serve as predictive markers for latent TB infection in the Iraq population. This study may result in a better method for identifying those more susceptible to the disease. The present study did have certain restrictions. The study's limitation was the use of relatively small sample sizes. Additional large-scale studies must thus confirm the prognostic usefulness of the SP110 SNPs identified in the present study.

Conclusion

The results found that genetic variants of the SP110 (rs9061, rs28930679) are associated with susceptibility to latent TB and single nucleotide polymorphism of the SP110 gene show the predictability of latent TB risk and subsequent TB

development. And therefore , rs9061 may be a predictive genetic marker for latent tuberculosis.

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