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The association between CCR5, NLRC4, and AIM2 gene polymorphisms and susceptibility to tuberculosis in the Iraqi population

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Abstract. *Khaleel B, Al-Alwani HRS, Asmar A. 2024. The association between CCR5, NLRC4, and AIM2 gene polymorphisms and susceptibility to tuberculosis in the Iraqi population. Biodiversitas 25: 1022-1029.* In immunological reactions against *Mycobacterium tuberculosis*, chemokines and their receptors, including CXCR3, CCR5, and RANTES, play an important role in the migration and activation of cells. The inflammasome is a multiprotein complex that serves as a master regulator of inflammation. Genetic polymorphisms contribute to individual phenotypic differences, such as disease risk and drug response. The current study was done to find the polymorphism in the CCR5, NLRC4, and AIM2 genes in our population and to look into how this polymorphism is linked to getting tuberculosis. Seventy-five tuberculosis patients participated in this study, while the control group comprised twenty-five control healthy individuals. We took two milliliters of blood from the patients and the control group and placed them in an Ethylenediaminetetraacetic acid (EDTA tube), or DNA extraction. Real-time PCR was used to find changes in the genes for CCR5 (rs2227010, rs2734648, rs1799987, rs1799988, rs1800023, rs1800024), NLRC4 (C-rs479333), and AIM2 (CC-rs1103577). Some genotypes were more common in the patient group, including SNPs CCR5 (rs2227010, rs2734648, rs1799988, rs1800023, rs1800024), NLRC4 C-rs479333, and AIM2 CC-rs1103577. However, by using Two-Two cross table the genotype of SNP CCR5-rs1799987 was not significantly different between the patient and the controls. Patients also had a lot of certain alleles for the SNPs CCR5 (rs2227010, rs1799988, rs1800023, rs1800024) and NLRC4 (rs479333). However, the alleles for the SNPs CCR5 (rs2734648, rs1799987) and AIM2 CC (rs1103577) were not very different between patients and controls. This study concludes that a polymorphism in various SNPs of the CCR5, NLRC4, and AIM2 genes may be a risk factor for tuberculosis.

Keywords: Chemokine's receptor (CCR5), gene polymorphisms, inflammasome (NLRC4, AIM2), Iraq, *Mycobacterium tuberculosis*

INTRODUCTION

Mycobacterium tuberculosis and *M. bovis* are the name of the bacterium that causes Tuberculosis (TB). Most *Mycobacterium TB* infections remain latent or undiagnosed, and only a tiny fraction of infected people develop active tuberculosis, according to Forrellad et al. (2020) and Ogishi et al. (2022). Inflammation is the immune system's first response to any danger, whether it is a disease, an irritation, or damaged cells. Chronic, or dysregulated, inflammation is a known disease initiator, Rheumatoid arthritis (Bennett et al. 2018). systemic lupus erythematosus, inflammatory bowel disease, metabolic problems, cardiovascular disease, neurodegenerative illness, and autoimmune diseases are all part of this category (Chen et al. 2018). A network of proteins is known as the inflammatory regulatory complex. Ordinary components include effector caspases, adaptor molecules, and Pattern Recognition Receptors (PRRs). Due to their PRRs and involvement in inflammasome activation, Non-Occlusive Disease (NOD)-Like Receptors (NLRs) have received the lion's share of study and attention (Davignon et al. 2023). Some examples of these are NLRP1, NLRP3, NLRC4, and many more. Inflammasomes rely on pyrin and the missing melanoma 2 (AIM2)-like receptor (AIM2), according to research by Zhou et al. (2021). A multiprotein complex called the inflammasome is essential to the innate immune system's reaction to microbial diseases, such as Tuberculosis. NLRC4 and AIM2, two inflammasome family members, have been linked to tuberculosis susceptibility (Ma et al. 2021). An intracellular sensor called NLRC4 initiates the inflammasome complex when it detects bacterial flagellin. According to a number of research, NLRC4 polymorphisms may be linked to TB susceptibility (De Lima et al. 2020). For instance, a study on the NLRC4 gene's CC genotype of the rs385076 C>T polymorphism indicated a higher risk of tuberculosis in those with this genotype in a Chinese population. A different study on a population in South Africa discovered that specific NLRC4 haplotypes were linked to a higher incidence of tuberculosis (Torres et al. 2019). An important part of the host's immune response to TB is the activation of T helper 1 (Th1) cells, which leads to more CCR5 being expressed (Blanco et al. 2021). Infections with TB are also associated with significantly increased CCR5 expression. Pulmonary TB patients show significantly higher levels of CCR5 expression compared to healthy controls (Liu et al. 2021). The ligand-CCR5 interaction has far-reaching effects on T cell activation and migration, the immunological response to TB infection, and other related processes. Research carried out by Fisher et al. (2021) indicates that effector CD8+ T cells without CCR5 transmigrate from the pulmonary vascular system to the interstitial compartment

at a rate about 50% lower than that of CD8+ T cells with CCR5 wild-type. Despite CCR5's critical role in resistance to *TB*, some variants in the gene have been associated with tuberculosis infections and disease development (Carpenter et al. 2014).

Barletta-Naveca et al. (2018) have studied the inherited vulnerability to TB. Fernandes et al. (2020) found that variations in inflammasome physiological activity that run in families may impact susceptibility, severity, and outcome in tuberculosis. According to de Lima et al. (2016), two Single-Nucleotide Polymorphisms (SNPs)-P2X7 (rs2230911) and NLRP3 (rs10754558)-are connected to TB. There is a connection between NLRP3 (rs10754558) and protection against pulmonary TB. A study by Abate et al. (2019) found that the SNP (rs35829419) has been associated with tuberculosis susceptibility in the Ethiopian population, whereas no significant associations were found with the P2X7 (rs2230911) and CARD8 (rs2043211) SNPs. SNPs are linked to tuberculosis susceptibility. The interaction of cytosolic NLR (leucine-rich repeat-containing, nucleotide-binding, and oligomerization domain) receptors, namely NLRP3 and NLRC4, with ligands enhances the function of the inflammasome. According to Zhang et al. (2017), studies conducted on dendritic cells and macrophages infected with *Mycobacterium tuberculosis* in vitro have primarily implicated NLRP3 in inflammasome activation. AIM2 lacks the HIN-200 DNA-binding protein necessary for cytosolic recognition of dsDNA, as noted by Kopitar-Jerala (2017). This protein has been extensively studied. This study aims to investigate whether mutations in the CCR5, NLRC4, or AIM2 genes are associated with tuberculosis risk in our population in Iraq.

MATERIALS AND METHODS

The study received approval from the National Center of Tuberculosis, Iraqi Ministry of Health, and the approval committee of the University of Anbar, Iraq. All participants provided informed written consent before sample collection. Based on the ethics of research, all the patients and control group gave thier consent for taking the blood and sputum samples.

The names of people identified with tuberculosis were selected, and they numbered (100). 75 patients with TB infections (pulmonary and extrapulmonary). Patients enrolled in the tuberculosis center were clinically diagnosed and included in the study. Twenty-five healthy individuals control without a history of infection made up the control group, were selected from our healthy population outside the hospital. All of the samples were from people in Baghdad and Ramadi City, Iraq. From August 2022 through January 2023, the samples were gathered under the supervision of medical specialists.

Procedures

The health conditions of the patients A blood anticoagulant tube was used to take two milliliters of peripheral venous blood. The presence of EDTA-K, or ethylenediamine tetraacetic acid, is included in this tube. For future reference, we stored the material at 4°C and extracted the DNA using the EasyPure® Genomic DNA Kit (TransGen, biotech. EE101-01) according to the manufacturer's instructions.

Prior to utilizing TE buffer as a blank solution, a Nanodrop spectrophotometer OneC (Thermo Fisher Scientific) equipped with basic computer software for data control and recording was employed to assess the quality of DNA samples. Two microliters of the isolated DNA were added for concentration measurement, yielding concentrations ranging from 63 to 84 ng/µL. Furthermore, the absorbance of DNA samples was measured at wavelengths of 260 and 280 nm using the Nanodrop spectrophotometer to ascertain purity. The A260/A280 ratio falling between 1.7 and 1.9 confirms the absence of contaminants in the DNA samples. We used Reverse Transcription and quantitative Polymerase Chain Reaction (qRT-PCR) to find out how much of the CCR5 polymorphism there was.

Utilizing the TransStart® Top Green qPCR Super Mix kit and the measurement of the threshold cycle (Ct), the levels and fold variations of the CCR5 polymorphism, NLRC4 C, and AIM2 CC genes were evaluated. It was necessary to repeat each response twice. The quantitative Real-Time-PCR (qRT-PCR) was performed using a QIAGEN Rotor gene Q real-time PCR system (Germany). The primers used for detection of gene polymorphisms in this study were instructed in the following Table 1.

Data analysis

Regarding statistical analysis, data were analyzed using SPSS for Windows, version 26 (SPSS Inc., Chicago, Illinois, United States). Results were presented as means and Standard Deviations (SD). The Shapiro-Wilk normality test was employed to verify the normal distribution of the data under study. Chi-square test was used to significantly compare between percentage (0.05 and 0.01 probability). Estimate of Odd ratio and CI in this study. WINPEPI and SPSS program were used to detect the genotyping.

Adds ratio

An odds ratio will be equal to 1, there is no connection between the SNP to the trait or genetic condition. In the other words, if the same percent of people in two groups have the SNP. An odds ratio greater than 1 means that having the SNP makes someone more likely to have the condition. It was found more often in the ALS group. We would consider that SNP a risk factor for that condition. An odds ratio of less than 1, means having the SNP makes some one less likely to have the condition. This would make the SNP protective factor for that condition.

Hardy Weinberg equilibrium of SNP

By comparing observed and expected value. If the p value is less than 0.05 then it implies is a deviation and you should look in to the causes of it. Deviance from HWE in control subjects may result from genotyping errors, from stratification of the control samples. Note: Be careful to test to test HWE on control only. Do not test on patients as a variant strongly associated with disease would sometime show deviance from HWE in patients' samples.

Table 1. Primers used for detection of gene polymorphisms in this study

Primer		Sequence (5′→3′ direction)	Primer size (bp)	Product size bp	Ta (°C)
CCR5 Polymorphism					
rs2227010 (A> G)	F	TGAGATTTTCAGATGTCACCA	21	48	58
	R	ACAGTCATATCAAGCTCTCTT	21		
rs2734648 (T>G)	F	CCCGTGAGCCCATAGTTAAAA	21	84	58
	R	ACAGATGCTCACCACCCAAT	20		
rs1799987 (G>A)	F	TTGGGGTGGGATAGGGGATA	20	74	58
	R	GGGGATCCTGGACTTCACAT	20		
rs1799988 (T>G)	F	CAAAATAATCCAGTGAGAAAAGC	23	110	60
	R	GAAAAGAATCAGAGAACAGTTC	22		
rs1800023 (G >A)	F	AGCCCGTAAATAAACCTTCAGAC	23	104	60
	R	AGTGTATTGAAGGCGAAAAGA	21		
rs1800024 (C> T)	F	AAGACTTTACAGGAAACCCA	20	104	60
	R	TCCAAACTGTGACCCTTTCC	20		
NLRC4 C (rs479333)					
Forward		ACTTGGGAGATTGGATGGACT	21	98	58
Reverse		GGAAACTCACTCTTGTTGCT	20		
AIM2 CC (RS1103577)					
Forward		ACTTCCACTACCTATCCCCT	20	103	58
Reverse		GCAAAGGGAAAAGGAGAGCC	20		

Note: If the p-value<0.05 that mean there is significant different or more prevalent or associated with disease based on each SNP result

RESULTS AND DISCUSSION

Results

Table 2 demonstrates that patients often have the genotypes GG and GA, as well as the alleles G and A, related to the CCR5 SNP (rs2227010).

There is a high prevalence of genotype GG and GT in patients when it comes to the CCR5-rs2734648 SNP. Table 3 shows that there is no statistically significant difference in the G and T alleles between the control group and the patients.

As shown in Table 4, there is no statistically significant difference in the genotypes and allele frequencies for the CCR5-rs1799987 SNP between the control group and the patients.

Table 5 shows that for the SNP CCR5-rs1799988, there was a high prevalence of the TT genotype and C, T alleles in the patients.

According to Table 6, a large percentage of patients had the AG, GG genotype and the A, G alleles for the SNP CCR5-rs1800023.

The result shows a high frequency of CC, CT, TT genotype, and C, T Alleles in patients for the SNP CCR5-rs1800024 as indicated by Table 7.

Table 8 displays the results for the NLRC4 C-rs479333 single-nucleotide polymorphism, which reveals that patients often had the AA genotype and the C and A alleles.

The results show a high frequency of genotype TA in the patients, while there is no significant difference in T, A alleles between patients and control, for the SNP of AIM2 CC-RS1103577 as indicated by Table 9.

Discussion

Mycobacterium tuberculosis is an infectious disease that poses a significant threat to human life. To comprehend why only a small percentage of individuals, ranging from 5% to 10%, exhibit symptoms of active TB infection (Klinkenberg et al. 2023). Researchers are striving to pinpoint associated risk factors. Understanding these factors is crucial for developing effective TB vaccines, immunotherapies, and unraveling the complexities of outcomes following *M. tuberculosis* infection, be it active or latent TB illness. Gathering data on host immune responses to *M. tuberculosis* infections is essential for these endeavors. There is substantial evidence suggesting that host genetics influence the progression of tuberculosis (Kauffman et al. 2018). CCR5 has been identified as playing a pivotal role in immunological responses to *Mycobacterium TB* infection, regulating T cell activation and macrophage recruitment (Liu et al. 2021). Various factors, both intrinsic and extrinsic to the human body, impact the immunological response to *Mycobacterium* TB. Despite this, little is understood about the intricate cascade of events from early infections to tuberculosis development. Following the elucidation of the diverse functions of inflammasomes, research has focused extensively on genetic variants within genes involved in assembling the inflammasome multiprotein complex. This pursuit aims to shed light on why certain individuals remain asymptomatic while others are predisposed to illness. During the initial stages of infection, the innate immune response assumes a pivotal role. According to the results, the ((GG, GA), (GG, GT), (TT), (AG, GG), (CC, CT, TT)) genotype is associated with a high frequency of certain CCR5 SNPs, including (rs2227010, rs2734648, rs1799988, rs1800023, rs1800024). This study's findings demonstrate that certain single-nucleotide polymorphisms (SNPs) in the CCR5 gene are associated with high frequencies of the ((G, A),(C, T),(A, G), and (C, T)) alleles in patients, namely (rs2227010, rs1799988, rs1800023, rs1800024).

Table 2. Frequency of genotype and CCR5 SNP rs2227010 gene alleles in patients and controls

CCR5-Rs2227010						
Genotype frequencies of SNP among patients and control						
Genotype Rs10	Patients no. () + (%)	Control no. + (%)	Odds ratio	95% CI	p-value	
GG	34 (45.33%)	23 (92.00%)	0.07	0.01 to 0.29	0.0001	
GA	32 (42.67%)	1 (4.00%)	17.86	3.01 to 381.87	0.0001	
AA	9 (12.00%)	1 (4.00%)	3.27	0.49 to 75.18	0.3	
Alleles frequency of SNP						
Alleles Rs10	Patients no.	Control No.	Odds ratio	95% CI	p-value	
G	100 (66.67%)	47 (94.00%)	0.13	0.03 to 0.39	0.0001	
A	50 (33.33%)	3 (6.00%)	7.83	2.54 to 32.92	0.0001	
Hardy Weinberg equilibrium of SNP						
Rs10		GG	GA	AA	X^2	p-value
Patients group	Observed genotype	34	32	9	0.12	0.9
	Expected genotype	33.33	33.33	8.33		
Control group	Observed genotype	23	1	1	10.41	0.005
	Expected genotype	22.09	2.82	0.09		

Note: *The mean difference is significant at p-value<0.05

Table 3. Genotype and alleles frequency of CCR5 SNP rs2734648 gene in patients and controls

CCR5-rs2734648						
Genotype frequencies of SNP among patients and control						
Genotype Rs48	Patients no. () + (%)	Control no. + (%)	Odds ratio	95% CI	p-value	
GG	24 (32.00%)	14 (56.00%)	0.37	0.14 to 0.95	0.04	
GT	27 (36.00%)	1 (4.00%)	13.5	2.27 to 290.22	0.001	
TT	24 (32.00%)	10 (40.00%)	0.71	0.28 to 1.86	0.4	
Alleles frequency of SNP						
Alleles Rs48	Patients no.	Control no.	Odds ratio	95% CI	p-value	
G	75 (50.00%)	29 (58.00%)	0.72	0.38 to 1.39	0.3	
T	75 (50.00%)	21 (42.00%)	1.38	0.72 to 2.66	0.3	
Hardy Weinberg equilibrium of SNP						
Rs48		GG	GT	TT	X^2	p-value
Patients group	Observed genotype	24	27	24	5.88	0.05
	Expected genotype	18.75	37.5	18.75		
Control group	Observed genotype	14	1	10	21.06	0.0001
	Expected genotype	8.41	12.18	4.41		

Note: *The mean difference is significant at p-value<0.05

Table 4. Genotype and alleles frequency of CCR5 SNP rs1799987 gene in patients and controls

CCR5-rs1799987						
Genotype frequencies of SNP among patients and control						
Genotype Rs87	Patients no. () + (%)	Control no. + (%)	Odds ratio	95% CI	p-value	
GG	29 (38.67%)	13 (52.00%)	0.58	0.23 to 1.47	0.2	
GA	42 (56.00%)	11 (44.00%)	1.62	0.64 to 4.12	0.3	
AA	4 (5.33%)	1 (4.00%)	1.35	0.16 to 34.78	0.7	
Alleles frequency of SNP						
Alleles Rs87	Patients no.	Control no.	Odds ratio	95% CI	p-value	
G	100 (66.67%)	37 (74.00%)	0.70	0.33 to 1.43	0.3	
A	50 (33.33%)	13 (26.00%)	1.42	0.70 to 2.99	0.3	
Hardy Weinberg equilibrium of SNP						
Rs87		GG	GA	AA	X^2	p-value
Patients group	Observed genotype	29	42	4	5.07	0.07
	Expected genotype	33.33	33.33	8.33		
Control group	Observed genotype	13	11	1	0.51	0.7
	Expected genotype	13.69	9.62	1.69		

Note: *The mean difference is significant at p-value<0.05

Table 5. Frequency of genotype and alleles of CCR5 SNP rs1799988 gene in patients and controls

CCR5-rs1799988						
Genotype frequencies of SNP among patients and control						
Genotype Rs88	Patients no.	Control no.	Odds ratio	95% CI	p-value	
CC	39 (52.00%)	16 (64.00%)	0.61	0.23 to 1.56	0.3	
CT	10 (13.33%)	6 (24.00%)	0.49	0.16 to 1.63	0.1	
TT	26 (34.67%)	3 (12.00%)	3.89	1.13 to 17.49	0.03	
Alleles frequency of SNP						
Alleles Rs88	Patients no.	Control no.	Odds ratio	95% CI	p-value	
C	88 (58.67%)	38 (76.00%)	0.45	0.21 to 0.92	0.02	
T	62 (41.33%)	12 (24.00%)	2.23	1.09 to 4.75	0.02	
Hardy Weinberg equilibrium of SNP						
Rs88		CC	CT	TT	X^2	p-value
Patients group	Observed genotype	39	10	26	39.43	0.0001
	Expected genotype	25.81	36.37	12.81		
Control group	Observed genotype	16	6	3	2.93	0.2
	Expected genotype	14.44	9.12	1.44		

Note: *The mean difference is significant at p-value<0.05

Table 6. Frequency of genotype and Alleles of CCR5 SNP rs1800023 gene in patients and controls

CCR5-rs1800023						
Genotype frequencies of SNP among patients and control						
Genotype RS23	Patients no.	Control no.	Odds ratio	95% CI	p-value	
AA	11 (14.67%)	5 (20.00%)	0.69	0.22 to 2.44	0.4	
AG	56 (74.67%)	6 (24.00%)	9.33	3.24 to 28.21	0.0001	
GG	8 (10.67%)	14 (56.00%)	0.09	0.03 to 0.28	0.0001	
Alleles frequency of SNP						
Alleles Rs23	Patients no.	Control no.	Odds ratio	95% CI	p-value	
A	78 (52.00%)	16 (32.00%)	2.30	1.17 to 4.60	0.01	
G	72 (48.00%)	34 (68.00%)	0.43	0.22 to 0.85	0.01	
Hardy Weinberg equilibrium of SNP						
Rs23		GG	GA	AA	X^2	p-value
Patients group	Observed genotype	11	56	8	18.43	0.0001
	Expected genotype	20.28	37.44	17.28		
Control group	Observed genotype	5	6	14	5.03	0.08
	Expected genotype	2.56	10.88	11.56		

Note: *The mean difference is significant at p-value<0.05

Table 7. Frequency of genotype and Alleles of CCR5 SNP rs1800024 gene in patients and controls

CCR5-rs1800024						
Genotype frequencies of SNP among patients and control						
Genotype RS24	Patients no.	Control no.	Odds ratio	95% CI	p-value	
CC	28 (37.33%)	22 (88.00%)	0.08	0.02 to 0.28	0.0001	
CT	24 (32.00%)	2 (8.00%)	5.41	1.32 to 36.03	0.01	
TT	23 (30.67%)	1 (4.00%)	10.62	1.77 to 229.58	0.004	
Alleles frequency of SNP						
Alleles Rs24	Patients no.	Control no.	Odds ratio	95% CI	p-value	
C	80 (53.33%)	46 (92.00%)	0.10	0.03 to 0.27	0.0001	
T	70 (46.67%)	4 (8.00%)	10.06	3.67 to 34.00	0.0001	
Hardy Weinberg equilibrium of SNP						
Rs24		CC	CT	TT	X2	p-value
Patients group	Observed genotype	28	24	23	9.57	0.008
	Expected genotype	21.33	37.33	16.33		
Control group	Observed genotype	22	2	1	5.21	0.07
	Expected genotype	21.16	3.68	0.16		

Note: *The mean difference is significant at p-value<0.05

Table 8. Frequency of genotype and Alleles of NLRC4 C SNP rs479333 gene in patients and controls

NLRC4 C-rs479333						
Genotype frequencies of SNP among patients and control						
Genotype Rs33	Patients no.	Control no.	Odds ratio	95% CI	p-value	
CC	31 (41.33%)	14 (56.00%)	0.55	0.22 to 1.40	0.2	
CA	21 (28.00%)	8 (32.00%)	0.83	0.31 to 2.31	0.7	
AA	23 (30.67%)	3 (12.00%)	3.24	0.94 to 14.67	0.05	
Alleles frequency of SNP						
Alleles Rs33	Patients no.	Control no.	Odds ratio	95% CI	p-value	
C	83 (55.33%)	36 (72.00%)	0.48	0.23 to 0.96	0.03	
A	67 (44.67%)	14 (28.00%)	2.08	1.04 to 4.26	0.03	
Hardy Weinberg equilibrium of SNP						
Rs33		CC	CA	AA	X^2	p-value
Patients group	Observed genotype	31	21	23	14.09	0.0009
	Expected genotype	22.96	37.07	14.96		
Control group	Observed genotype	14	8	3	1.06	0.5
	Expected genotype	12.96	10.08	1.96		

Note: *The mean difference is significant at p-value<0.05

Table 9. Frequency of genotype and Alleles of AIM2 CC SNP rs1103577gene in patients and controls

AIM2 CC-RS1103577						
Genotype frequencies of SNP among patients and control						
Genotype Rs77	Patients no.	Control no.	Odds ratio	95% CI	p-value	
TT	33 (44.00%)	7 (28.00%)	0.02	0.76 to 5.71	0.2	
TA	28 (37.33%)	17 (68.00%)	0.28	0.10 to 0.74	0.008	
AA	14 (18.67%)	1 (4.00%)	5.51	0.88 to 122.19	0.07	
Alleles frequency of SNP						
Alleles Rs77	Patients no.	Control no.	Odds ratio	95% CI	p-value	
T	94 (62.67%)	31 (62.00%)	1.03	0.52 to 1.99	0.9	
A	56 (37.33%)	19 (38.00%)	0.97	0.50 to 1.91	0.9	
Hardy Weinberg equilibrium of SNP						
Rs77		TT	TA	AA	X^2	p-value
Patients group	Observed genotype	33	28	14	3.06	0.2
	Expected genotype	29.45	35.09	10.45		
Control group	Observed genotype	7	17	1	4.909	0.08
	Expected genotype	9.61	11.78	3.61		

Note:*The mean difference is significant at p-value<0.05

The study also observed no statistically significant difference in the genotypes or allele frequencies of the CCR5-rs1799987 SNP between the control group and the patients. Similarly, for the CCR5 SNP, rs2734648, there was no statistically significant difference in the G and T alleles between the patient and control groups. A notable correlation between TB recurrence and rs2734648 was identified. Mutations in CCR5 have been demonstrated to alter chemokine responses, particularly in how the receptor binds to ligands (Liu et al. 2021). Among tuberculosis patients, the PTB group exhibited a significantly higher allelic rs2734648-G frequency in the recessive inheritance model compared to the control group, as reported by Shuyuan Liu et al. (2021). Individuals carrying rs2734648-GG were found to have a 2.382-fold increased risk of PTB exposure (Liu et al. 2021). Additionally, according to their findings, under a recessive inheritance scenario, rs1799987-AA increases the likelihood of PTB susceptibility. The variant rs1799987 (2459A>G) affects CCR5 production, as outlined by Jasinska et al. (2022). In vitro studies have shown that rs1799987-G exhibits a 45% reduction in promoter activity compared to rs1799987-A. The CCR5 promoter demonstrates significant linkage disequilibrium, and investigations into promoter haplotypes in relation to tuberculosis susceptibility have only recently begun (Kouhpayeh et al. 2016). It is possible that other receptors with overlapping specificities may fulfill similar functions as CCR5, which could explain the lack of association with the CCR5 polymorphism. The function of macrophage inflammatory protein-1 alpha (MIP-1α/CCL3) involves interaction with receptors CCR1 and CCR3 on various immune cells. Mice lacking CCR5 are capable of forming granulomas when infected with Mycobacterium TB, maintaining infection control, and initiating a Th1 response (Domingo-Gonzalez et al. 2016). Promoter SNPs are crucial for CCR5-mediated protection against TB. However, it has not been demonstrated that SNP associations with diseases are consistent across different populations.

The dominant allele Rs2734648-G is present in almost every human population on Earth. This includes almost every South Asian, European, American, and African person. To learn how rs2227010 and rs1800024 influence *M. tuberculosis* infection and TB progression, more functional studies are required.

Compared to other allelic combinations of rs1799987/ rs1799988, rs1799987-A/rs1799988-C boosts CCR5 activity as a promoter by 45%, according to subsequent authors (Jasinska et al. 2022). Li et al. (2005) found that the rs1799988 C-to-T mutation, which leads to a reduction in CCR5 expression, is associated with a slower course of Acquired Immunodeficiency Syndrome (AIDS). Different from the other two CCR5-rs1799988 genotypes, rs1799988-CC carriers have more CCR5 transcription on CD4+ cells, CD4+ monocytes, and peripheral blood mononuclear cells (PBMCs).

Han Chinese people infected with *Mycobacterium tuberculosis* (*Mtb*) have eight different variations in the promoter region of the CCR5 gene. The rs1799987-AA and rs2734648-G genotypes were linked to an increased risk of TB, or pulmonary tuberculosis, at both the single and double dose levels. Chromosome 5 variations: rs2227010A, rs2734648G, rs2856758A, rs1799987G, rs1799988T, rs1800023G, rs41469351C, and rs1800024C. Analysis of the eight variations' haplotypes showed an elevated risk of tuberculosis in the lungs and TB vulnerability (Harishankar et al. 2018). The promoter polymorphism of the CCR5 gene was shown to be associated with pulmonary TB and its development in the Iraqi population, according to this research. Patients in Iraq who had contracted *Mtb* were examined for these variations. Those who had the rs2227010, rs2734648, rs1799988, rs1800023, or rs1800024 genotypes were more likely to be susceptible to TB or asthma.

Patients with the (AA) genotype and (C, A) alleles are more likely to have the NLRC4 C-rs479333 SNP, according to this research. On top of that, the study discovered that a lot of patients had the (TA, AA) genotype for the AIM2 CC-rs1103577 SNP. There was no significant difference in the frequency of the alleles for this SNP between the patients and the controls.

According to research by De Lima et al. (2020), extra-pulmonary tuberculosis is associated with at least one NLRC4 C (rs479333) allele, which indicates a significant increase in tuberculosis susceptibility. In regions where TB is prevalent, NLRC4 is linked to the outcome of the disease (De Lima et al. 2020). People with extra-pulmonary TB were less likely than people with pulmonary illness to have the functional mutation lacking rs479333 in the NLRC4 gene (49% of those who had it).

The risk of getting pulmonary TB was shown to be greater in individuals with the AIM2 CC (rs1103577) genotype compared to non-carriers, according to research by de Andrade Figueira et al. (2021). A decreased risk of the CTSB gene (rs1692816) was associated with extrapulmonary symptoms. The association between Tuberculosis (TB) protection and a single nucleotide variation (SNV) in AIM2 (rs1103577) was found by Yan et al. (2018). Immunological studies in *Mus musculus* (mice) have shown that AIM2 has a novel protective function against *Mycobacterium tuberculosis* infection. It is thought that the AIM2-inflammasome and NLRP3 are crucial in host defence against mycobacteria. Multiple studies have shown that the AIM2-inflammasome is involved in mycobacterial infection. Macrophages infected with the pathogenic strain of *Mycobacterium bovis* generated mature IL-1β and activated the AIM2-inflammasome (Yang et al. 2013; de Andrade Figueira et al. 2021). When it came to the correlation between AIM2 and NLRC4 and the increased risk of TB development, this research was in line with other studies.

Accordingly, further studies with larger sample sizes of people from different demographics are necessary. Furthermore, as previously stated, SNP combinations have a pivotal role in tuberculosis susceptibility and progression.

In conclusion, the genetic variation in one or more SNPs of the CCR5, NLRC4, and AIM2 genes in Iraq population may be considered a risk factor for individuals to be susceptible to *Mycobacterium tuberculosis*. The probable role of CCR5, NLRC4 and AIM2 involved in immune response against *Mycobacterium tuberculosis* indicates that any genetic variation in above-mentioned genes may be involved in susceptibility to tuberculosis.

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