Research Article

World Journal on Immunology

Investigating The Function Of Single-Nucleotide Polymorphism Of C-C Motif Chemokine Ligand-2 In Pulmonary Tuberculosis: A Genetic Association Study From North India

Sanjay K. Biswas[1], Mayank Mittal[1], Ekata Sinha[1], Vandana Singh[1] and Nidhi Arela[1]

Department of Surgery.

Corresponding author:
Sanjay K,
Department of Surgery.

Received Date: 01 April 2024
Accepted Date: 16 April 2024
Published Date: 22 April 2024

Citation:
Sanjay K. Investigating The Function Of Single-Nucleotide Polymorphism Of C-C Motif Chemokine Ligand-2 In Pulmonary Tuberculosis: A Genetic Association Study From North India. World Journal on Immunology 2024.

1. Academic Editor: Paulina Wlasiuk

2. Introduction

Globally, tuberculosis (TB) is a serious health hazard. Globally, between 5 and 500 cases of tuberculosis per 100,000 persons were reported in 2018. Of these, 57% were males, 32% were women, and 11% were children under the age of 15; 1.2 million of these cases resulted in TB-related deaths [1]. Eight nations geographicallyHindawi Journal of Immunological Research Volume 2020, Page 11; Article ID 1019639The doi:10.1555/2020/1019639 is the link.represented two thirds of the world's tuberculosis cases, with South Africa having the lowest rate at 3% and India having the highest rate at 27% (the 2019 edition of the global TB report was released on October 17, 2019). (www.who.int/tb/data). The intricate process of susceptibility to infectious diseases following pathogen exposure includes interactions between the host, pathogens, and environmental factors[2]. Numerous investigations have confirmed that hostgenetic variables play a critical role in PTB susceptibility [3]. Our first line of defense activates during exposure to M. tuberculosis, triggering adaptive immunity mostly driven by CD4+ T cells and macrophages, with assistance from a network of inflammatory cytokines (TNF-α and IFN-γ) andChemokines. Chemokines are tiny molecular weight proteins with roles in immunoregulatory and inflammatory processes [4]. They are divided into the following groups according to the cysteine residues at the N-terminus of their proteins: Subfamilies C–, C–, C–X–, and C–X3–C [5]. TNF-α is known to increase CCL2, a potent chemotactic and proinflammatory chemokine that is part of the C-C family. It has been shown to protect against M. tuberculosis [6] and to activate macrophages [7-8]. Human chromosome 17q11–17q12 contains the chemokine gene.

Two polymorphisms, -2518 A>G (rs 1024611) and -362G/C (rs 2857656), have been identified in the promoter region; both mutations impact gene expression and have been associated with tuberculosis susceptibility [9].Numerous investigations over the globe have been carried out to comprehend the impact of mutations in these variations on the susceptibility or resistance to pulmonary tuberculosis (PTB). The first investigation in this regard was carried out in a Mexican population by Flores-Villanueva, who found that carriers of the AG and GG gene types had 2.3- and 5.4-fold higher probabilities of acquiring pulmonary tuberculosis, respectively, than those who were homozygous AA. Additionally, they stated that GG had the lowest level of plasma IL-12p40 and the greatest level of plasma CCL2 [10]. According to a population study on people from Ghana and Russia, control groups had higher prevalence of -2518G and -362C than PTB groups did.instances, showing the alleles' protective effect against PTB sickness in a Ghanaian community; however, they found no relationship in a Russian sample [11]. A different study from Mexico and Peru found that the combination of the MMP1-1607GG genotype and the CCL2-2518GG genotype raised the chance of developing PTB by 3.9 in a Peruvian population and 3.59 in a Mexican population, respectively [12]. Higher prevalence of the CCL2-2518G variant was reported by Arji et al. [13] in a healthy Moroccan population, indicating that the allele may have a protective effect.in opposition to PTB illness. According to a meta-analysis by Gonget al. [14], the C allele of the -362G>C polymorphism is a protective factor against tuberculosis in these populations, while the G allele of the CCL2-2518 polymorphism is a risk factor for PTB in Asians and Americans but not in Africans. The -2518A>G and -362G>C polymorphisms were examined in PTB cases and healthy controls in a study done on the Sahariya tribe in India, but no correlation with PTB disease was found [15]. An further study from aThe CCL2-2518GG genotype was shown to be much less common in male PTB patients and significantly more common in female PTB patients, according to data from the South Indian population. According to their findings, the -2518GG genotype may be linked to PTB susceptibility in females and protection in males[16]. A recent meta-analysis revealed a correlation between human tuberculosis susceptibility and the CCL2-2518A>G polymorphism [17]. Previous research on populations from South Africa [19] and Hong Kong [18] was unable todiscover a meaningful correlation with the illness. It is well known that the two CCL2 polymorphisms (-2518A>G and -362G>C) that are found in the gene's promoter region are significant for the control of immune genes. We examined these polymorphisms in the north Indian population from Agra, India, because of the divergence in previous global findings and in an Indian population.Thus, the current study was carried out with two primary goals in mind: first, to investigate the relationship between TB and the CCL2-2518A>G and -362 G>C polymorphisms and haplotypes in a population from northern India; second, to examine the relationship between the levels of serum CCL2 and cytokines in TB cases and controls in relation to their genotypes.

3. Materials and Methods

Study Participants. The current study was carried out as a component of a larger project being carried out at the institute, which was approved by the human ethics committee of the institute and formed in accordance with the rules established by the Indian Council of Medical Research, New Delhi [20]. Prior to the study's commencement, an interview schedule was developed that included written informed consent and the demo-graphic details of the cases and controls. The institute's ethical council also approved of this plan. All study participants provided written informed consent, and in the case of minors or children under the age of 18, the consent was obtained from a parent or legal guardian.The study comprised 248 healthy controls (mean age 33:71 ± 12:82; male: female 122: 126) and 373 pulmonary tuberculosis patients (PTB) (mean age 32:47 ±12:94; male: female 253: 120). We examined the G>C polymorphism in CCL2-362 G>C in 330 PTB cases and 235 healthy controls, and the A>G polymorphism in CCL2-2518 in 373 PTB cases and 248 healthy controls. Using the interview schedule for both the cases and controls, we were able to gather data on the subjects' age, sex, drinking and smoking patterns, and BCG vaccination history.Patients with pulmonary tuberculosis (PTB). Patients with pulmonary tuberculosis (TB) between the ages of 16 and 63 were included in the study. PTB cases were selected from the State Tuberculosis Demonstration Centre (STDC), Agra, outpatient department (OPD) between 2007 and 2012. Participants in the study had to agree to participate and meet the inclusion and exclusion criteria of the PTB cases in order to be registered in the OPD on Monday, Wednesday, and Friday.

Most of the incidents were people who lived in or close to Agra.2. Journal of Research in Immunologyregion, all of Uttar Pradesh state. Specific clinical criteria, such as the common respiratory symptoms (fever, cough, expectoration, and malaise), were used to recruit the cases. Based on acid fast bacillus (AFB) smear positivity by Zeil-Neelsen staining and clinical symptoms, the sputum smear and/or culture positivity were diagnosed in accordance with the Revised National TBControl Programme (RNTCP) guidelines [21]. Two sputum samples were routinely taken over the course of two days (on spot/morning sputum), and a new smear-positive pulmonary tuberculosis case was only diagnosed when any of the sputum samples produced a smear-positive result. The culture of AFB wasdeveloped in Lowenstein-Jensen (LJ) slant, and M. tuberculosis was verified using biochemical testing in accordance with Vestal's [22] guidelines. All cases exhibiting symptoms of another type of tuberculosis, those who tested positive for HIV, those with immunosuppressive conditions such as diabetes mellitus, and those who had previously taken anti-TB medications were excluded from the study.wholesome controls. A house-to-house survey method was used to randomly select healthy subjects who were between the ages of 16 and 63 who were escorting PTB cases to the hospital and did not have a blood connection to the cases. Postgraduate students who were short-term trainees in the institute and who agreed to participate in the study and met the inclusion criteria of controls were also included as healthy controls. Individuals who have recently experienced a fever, viral infection, other sickness, or any other immune-related condition, and who have undergoneIndividuals who had received treatment for leprosy or TB in the past, had a family history of tuberculosis, or tested positive for AFB smear tests were not allowed to participate in the study. After 0.1 ml (5 tuberculin units) of PPDantigen was applied intradermally to 104 healthy controls, induration was

observed 48 to 72 hours later. Of these, 34 (32.69%) tested positive for PPD and 70 (67.30%) tested negative. A thorough explanation of PTB cases and healthy controls may be found in.DNA extraction and blood sample collection. Each patient gave a total of 4 milliliters of blood, of which 2 milliliters were taken and placed in tubes containing acid citrate dextrose (ACD). From this, DNA was separated using the DNA isolation kit (Midi prep from Qia-gen, Germany) in accordance with the user's instructions. In order to separate the serum, another 2 milliliters of blood were drawn into tubes devoid of anticoagulants. The separated serum was then refrigerated at -20°C with a protease inhibitor for assays measuring serum cytokines such as CCL2. Choosing the Sample Size and Single Nucleotide Polymorphism (SNP). There have been reports linking the SNPs CCL2-2518 A>G and -362 G>C to TB susceptibility or resistance in a number of global groups [6–19]. However, a discrepancy in the data that has been reported and the fact that the two polymorphisms of CCL2-2518A>G and -362G>Clocated in the gene's promoter region, which is crucial for immune regulatory mechanisms, led us to examine the polymorphism in the population of north India.tion from India's Agra. Therefore, the goal of the current study was to examine the relationship between TB and the polymorphisms CCL2-2518 A>G and -362 G>C, as well as the levels of serum CCL2 and cytokines, in a population from northern India. After a tiny pilot study with a modest sample size was first conducted and positive results were discovered, the sample size was determined using statistical techniques. enotyping of single-nucleotide polymorphisms -362 G>C and -2518 A>G.

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach, previously published by Flores-Villanueva [10], was used to genotype the CCL2-2518A>G polymorphism. Using 100 ng of genomic DNA, the region containing the CCL2 promoter region's -2518 A>G polymorphism was amplified using the forward primer 5′-GCTCCGGGCCCAGTATCT-3′ and the reverse primer 5′-ACAGGG.3′ AAGGTGAAGGGTATGA. The CCL2 alleles were found using the restriction enzyme PvuII. The presence of an undigested fragment measuring 236 bp identified allele A, while two fragments measuring 182 bp and 54 bp each were produced following digestion for allele G. The agarose gel electrophoresis was used to resolve these fragments.Melting curve analysis with fluorescence-labeled hybridization probes (TIB Mol Biol, Berlin, Germany) was used to genotype for the -362G>C polymorphism. Using the modified technique of Thye et al. [11], the Light Cycler 480 system (Roche Diagnostics, Berlin, Germany) is utilized.FRET probes, along with the sense primer 5′-GAGCCTGACATGCTTTCATCTA-3′ and the antisense primer 5′-TTTCCATTCACTGCTGAGAC-3′, Fluorescein was used to label 5′-TTCGCTTCACAGAAAGCAGAATCCTTA-3′ and 5′-AAATAACCCTCTTAGTTCACATCTGTGGTCAGTCT-3′ (5′ labeled with LCRed640). 1.5 µl sense and antisense DNA primers were used in the PCR, along with 1.25 pmol, 2.5 mM MgCl2, and 250 nM of the sensor and anchor probes. Fluorescein was used to mark the sensor probe's 3′ end. At the 5′ end of the anchor probe, Light CyclerRed 640 was written. Different homozygous and heterozygous genotypes were identified by differences in melting peak temperatures.

Not a Ordering. Using sequence-specific primers previously reported [10, 11], the region covering both polymorphisms of the CCL2 gene, -2518 A>G and -362 G>C, was amplified in 10 samples from each genotype using the ABI Big Dye Terminator v2 kit (Applied Biosystems, Foster City, CA, USA) in conjunction with the ABI-recommended protocol in the ABI 3700 capillary sequencer.CCL2, IL-12p70, IFN-γ, TNF-γ, and TGF-β estimation. Using the appropriate human Duosetenzyme-linked immunosorbent assay (ELISA) DevelopmentSystem (R&D Systems, Minneapolis, MN, USA), serum levels of CCL2, IL-12p70, IFN-γ, TNF-γ, and TGF-β were measured in 120 tuberculosis cases (40 representative cases each from wild, heterozygous, and mutant genotypes) and 54 healthy controls (20 representative healthy controls from each of the wild and heterozygous genotypes and 14 from mutant genotypes with regard to the CCL2-2518A>G polymorphism). Analytical Statistics. Direct counting was used to ascertain each polymorphism's genotype and allele frequencies.The $\chi 2$ test was used to assess the Hardy-Weinberg equilibrium (HWE) in patients and controls. The chi-squared test was used to compare the genotype and allele frequencies of the patients and controls; the strength of the link was reported as an odds ratio (OR) with a 95% confidence interval. For every analysis, a significance level of $p < 0:05$ was held. STA-TA/MP16.1 software (StataCorp LP Lakeway Drive, CollegeStation, TX, USA) was used to test genotypic associations for dominant, recessive, and overdominant models. After sex adjustment, the Mantel-Haenszel (M-H) estimate was also computed. M-Hestimates as well as crude estimates were given. Using online softwareSNP stats, linkage disequilibrium (LD) and haplotype analysis between the SNPs were performed. The Mann-Whitney or Kruskal-Wallis tests were used to compare the levels of serum CCL2 and cytokines.Using STATA/SE 11.0 software, the Spearman rank correlation test was carried out.

4. Results

Analysis of Demographic Parameters. In this study, 248 healthy controls (HCs) aged 16 to 63 and 373 PTBcases respectively were included. The male to female ratio was found to be substantially larger in PTB cases compared to HCs ($p = 0:007$), while the mean age of HCs (33.71) and PTB cases (32.47) did not differ significantly. (Table 1).AFB smear positive could be examined in 370 of the 373 PTB patients, and AFB culture positivity in 368 of the cases. Table 1 depicts the precise distribution of AFB smear positivity and culture positivity. The PTB cases underwent additional analysis based on the bacterial burden (scanty +, 1+).2+, and 3+) as well as for any notable variations in age, gender, and polymorphism; however, no noteworthy variations or correlations were discovered.When the PTB illness was examined on healthy controls as well, none of them displayed any indication of AFB smear positivity or culture positivity. PPD (purified protein derivative) could be administered to 104 of the 248 HCs; of them, 32.69% were determined to be PPD positive and 67.30% to be PPD negative.HCs with positive PPD were monitored throughout the trial, and none of them experienced the development of PTB in an active form.Single nucleotide polymorphisms CCL2-2518 A>G (rs1024611) and 362 G>C (rs2857656) were analyzed genotypically.The CCL2-2518A/G polymorphism was examined in 248 HCs and 373 PTBcases. In all cases and controls, the genotype and allele frequencies were in

Hardy-Weinberg equilibrium (p > 0:05). When comparing PTB cases to healthy controls, the male to female ratio is noticeably larger; yet, there is no statistically significant difference in the frequencies of genotypes for CCL2-2518A>G and 362G>C between males and females (p = 0:81 and 0.93). There was a notable difference in the genotypic frequencies of PTB cases and healthy controls.The homozygous GG genotype was shown to be significantly greater in PTB patients (0.11, p = 0:004), but the heterozygous AG genotype was found to be significantly higher in controls (0.43, p < 0:003). An allele with frequencies of 0.73 and 0.72 in the patients and controls, respectively, was determined to be the dominant allele. There was no significant difference between them (p = 0.79). After noting the statistically significant variation in genotypic frequencies, we examined the genotype relationship.with disease utilizing a variety of models, and found that the heterozygous AG genotype was offering resistance against PTB disease in the dominant model [OR = 0:60(95%CI = 0:43-0.84), p value = 0.003]. However, the homozygous recessive genotype GG in the recessive model demonstrated an almost twofold increased risk of the disease [OR = 1:97 (95%CI = 1:06-3.64), p value = 0.02] and M-estimates after sex adjustment [OR = 2:07 (CI = 1:10-3.91)].The CCL2-362G>C polymorphism was examined in 235 HCs and 330 PTBcases. In both PTB cases and healthy controls, the polymorphism was in Hardy-Weinberg equilibrium (p > 0:05). PTB sufferers and healthy controls differed significantly in the frequency of homozygous CC and heterozygous GC genotypes for the CCL2-362 G>C polymorphism (rs2857656). In PTB cases, there was a significantly greater frequency of homozygous CC genotype at locus -362G>C (0.13) compared to that4 Journal of Research in Immunology

5. Discussion

In the current study, a north Indian population from Agra, India, was examined for the genetic frequencies of the CCL2-2518 A>G and -362 G>C polymorphisms. Healthy controls and pulmonary tuberculosis cases were selected with consideration for their comparable socioeconomic status and environmental exposure.While several reports have been released indicating the significance of CCL2 gene variation in various populationstions, the results are frequently incongruous depending on the population [24], ethnicity [23], and kind of tuberculosis [25, 26]. Additional research from India is centered on tribal populations [15] and south Indian populations [27]. Our research is based on a sample of people from the northern region of India, and there is currently no information on the CCL2 gene polymorphism in this area in relation to tuberculosis. Because tuberculosis is common in this area, the study's participants were recruited between 2007 and 2012. This provided a compelling reason for the study's execution. We have made an effort to partially address the functional importance of this polymorphism in relation to its potential regulatory role in cytokine levels in comparison to other investigations. Based on our observations, the prevailing allele and genotype in the current population are identified as the -2518A allele and the -2518AA genotype, respectively. In a Mexican population, Flores-Villanueva et al. identified a GG genotype and the -2518 G allele tobe the predominant genotype and allele in their investigation, respectively[10]. They showed that carriers of the GG genotype had a 5.4% and 6.9% higher risk of contracting tuberculosis in the Mexican and Korean populations, respectively; we also discovered the similar link between the GG genotype and the CCL2-2518 gene.with TB susceptibility in the recessive model (p = 0:02) (Table 2). In contrast to their findings, which showed a 2.3- and 2.8-fold greater chance of contracting the disease in a Mexican and Korean population, respectively, our analysis of the AG genotype revealed that -2518 AG was offering resistance to the illness.Value of p (0.003) in Table 2. The G allele of the -2518 polymorphism has also been linked to associations in other demographic studies. Gon et al.'s meta-analysis [14] found that the G allele of the -2518 polymorphism is associated with a higher risk of tuberculosis in Asian and American populations, but not in African populations. The protective function of the G allele of the -2518 polymorphism in a Ghanian and Moroccan population, respectively, has been documented by Thye et al. [11] and Arji et al. [13]. A study on the Indian Sahariya tribe, who is known to have a high prevalence of tuberculosis, found no evidence of a relationship with the CCL2-2518A/G polymorphism [15]. An additional study on a South Indian population conducted on the Indian mainlandfound that the CCL2-2518 GG genotype is linked to protection against PTB in males, but it is also linked to a higher risk of contracting the disease in females [16].

The GG genotype of -2518 was shown to have a roughly 2-fold greater risk of developing PTB in the current investigation.In the current investigation, we discovered that the CCL2-362 GC geno-type was substantially higher among healthy controls (p = 0:01) than the other variant of CCL2 that has been described globally, which is -362G>C.compared to PTB cases, suggesting a protective role of the genotype against PTB; however, the homozygous-362 CC genotype is found to be a risk genotype (Table 2); this is in contrast to the findings of Thye et al. [11], who reported that the 362C allele was linked to protection against TB and that both the CC and CG genotypes were overrepresented in healthy controls in a Ghanaian population. Mishra et al. [15] were unable to identify any genotype or allele frequencies of the -362 G>C polymorphism that differed significantly among thePTB cases and HCs of the "Saharia" tribe, a primitive tribal group; nonetheless, controls had a higher frequency of the GC genotype. Additionally, Velez Edwards et al. [28] were unable to discover any proof linking the -362 G>C polymorphism to the populations of African Americans, Gambians, or Guinea Bissau. In a meta-analysis of five case control studies from five different ethnicities, Thye et al. found a significant heterogeneity in the association between the -362 G>C polymorphism and PTB between studies [11]. The disparity in observations could be due to the ethnic variance.The population including multireligious communities living in the vicinity of Agra in Uttar Pradesh and neighboring states was the source of our study participants. Here, we discovered that PTB patients are overrepresented in the GG genotype of CCL2 2518 A>G. In contrast to PTB patients, the AG genotype is greater in healthy controls (both tuberculin-positive and tuberculin-negative controls). Nothing changed.differences in the genotype frequencies of those who are tuberculin-positive and tuberculin-negative, and the national frequency for PPD (+) and PPD (-) individuals. The fact that the heterozygous genotype protects against the illness is remarkable. In

a heterozygous state, both the polymorphisms -2518 A>G and -362 G>C provide protection against the illness.The over-dominance hypothesis, which represents heterozygous protection, postulates that polymorphism is preserved because heterozygous people can identify a greater range of parasites [29]. Since India is an endemic place for tuberculosis, we can hypothesize thatThis heterozygous effect was previously reported by Sinha et al. [30] and may have contributed to local adaptation against tuberculosis. We further examined the functional component of CCL2 in serum to support our theory. In line with previous research findings [4, 5], we found a considerably increased level of serum CCL2 in PTB patients when compared to healthy controls.

Our results show a high correlation between the serum CCL2 level and different genotypes of the -2518A>G polymorphism in PTB cases, which contradicts their observations. Serum CCL2 levels were significantly greater in PTB subjects with the -2518AA genotype than in cases with the -2518 AG and2518 genotypes of GG. Patients with 2518 GG had lower serum IL-12 levels and a greater level of CCL2, according to Flores-Villanueva et al. [10] and Rovin et al. [31]. The serum levels of IFN-γ and IL-12p70 were found to be considerably greater in PTB sufferers as compared to healthy controls. In both study subject groups, there was a positive correlation between the levels of IL-12p70 and CCL2. In PTB cases with the -2518 GG genotype, there was a significant correlation between the level of CCL2 and IL-12, IFN-γ, and TNF-α. Therefore, it is possible that the decreased levels of CCL2 are what caused the apparent low levels of IL-12 and IFN gamma.resistant cytokines that provide TB infection resistance. This could be the cause of the current population's -2518 GG genotype vulnerability to PTB. Less CCL2 and IL-12p70 were created by -2518 GG genotypecases, which elevated their vulnerability to the illness.Therefore, in the current population, patients with -2518 GG genotypes are more likely to be susceptible to M. tuberculosis infection, and their lowered levels of IL-12p70 may have an impact on their weakened immunity. FurthermoreIn addition, healthy individuals with AG genotypes exhibiting an inter-mediate level of IL-12p70 most likely regulate the CCL2 level in some manner, providing the current population's protection against M. tuberculosis infection.

The current study also examined, for the first time, the levels of other significant cytokines in serum in relation to different CCL2-2518 A>G genotypes.PTB participants with the genotype -2518GG were experiencinga reduced level of IFN-γ, most likely as a result of decreased CCL2 and IL12p70. Compared to PTB cases, the TGF-β level was considerably greater in healthy controls. The increased concentration of TGF-β in individuals in good health who have the 2518AG genotype suggests that it has a regulatory role in preventing tuberculosis infection. We investigated the relationship between serum CCL2 levels andL-12p70, IFN-γ, TNF-α, and TGF-β in individuals with -2518A>G variants. A regression study revealed that the concentration of theserum CCL2 controls the levels of IL-12p70 and, to a greater extent, IFN-γ. Genotype-based stratification revealed that in healthyProtective immunity may be provided by individuals with the -2518AA or -2518AG genotype who have high levels of CCL2, since they likely positively influence the production of important cytokines like IFN-γ and IL-12p70. However, PTB subjects with the CCL2-2518 GG genotype had lower CCL2 concentrations, which decreased IFN-γ production and made them more vulnerable to8 Journal of Research in Immunologyinadequate Th1 response leading to infection.

In a previous study, Velez Edwards et al. [28] noted that there was an opposite effect in Africans when one of the CCL2 and IL12B polymorphisms interacted.Higher CCL2 was generally thought to stimulate Th2 response while suppressing Th1 response. For the first time, the current study demonstrated a positive association between CCL2 and Th1 cytokines including IFN-γ and IL-12.together with TNF-α, making them necessary for a healthy Th1 response against TB. The -2518G gene is in charge of reducing CCL2 synthesis, which in turn results in fewer Th1 cytokines. This faulty Th1 response leaves a host vulnerable to tuberculosis. There have been several well-reported findings about the genetic correlation between various diseases in populations with diverse ethnic backgrounds. Similar nucleotide polymorphisms can influence susceptibility in a variety of ways depending on the environmental conditions. Thesecomprise the length of time spent exposed to infectious pathogens, the people's nutritional status, and additional epigenetic variables. The diverse observations may also be caused by the population's genetic variations and the database's relatively limited size.CCL2, or C-C motif chemokine ligand 2, is a member of the SIG family of small inducible genes. CC-chemokines are identified by the presence of two neighboring cysteine residues next tothe molecule's amino terminus. They have a role in the recruitment of monocytes and lymphocytes as well as the regulation of these cells' movement to areas of cellular damage and immunological responses [32]. Various cell types create CCL2 in response to microbiological stimuli [33]. Studying the haplotypic analysis of the polymorphism is crucial because the polymorphisms examined in the current population may have an impact on one another. In the current investigation, AGThe most common haplotype in both PTB sufferers and healthy controls was discovered to be haplotype. It's interesting to note that according to haplotype analysis, the AC haplotype is susceptible to tuberculosis (Table 3). Strong D′ existed between the two polymor-phisms (-2518 A>G and -362 G>C) in a linkagedisequilibrium.

Through interaction analysis, Thye et al.[11] have demonstrated that the While CCL2 -2518A>G was not independent of CCL2 -362 G>C, it does explain the observed link with resistance to tuberculosis solely. These observations imply that this haplotype block, which is made up of these two polymorphisms, has been jointly inherited in the current population and may have a specific impact on the development of tuberculosis under the current environmental conditions. inteMore protection is provided by 2581G/-362C/int1del 554-567 than by the -362 G>C variation alone. Both the risk of tuberculosis and CCL2 expression are reduced by these haplotype variants. According to Ganachari et al. [12], the haplotype containing CCL2-2518 GG and MMP-1607 GG raises the risk of tuberculosis (TB) -3.5 times in Mexican populations and 3.9 times in Peruvian populations.The susceptibility of M. tuberculosis infection in this region was shown to be associated with the AC haplotype of CCL2-2518 A>G and -362 G>C. This suggests that polymorphisms in these

regulatory regions may have a complex role in impacting both disease susceptibility and immune function. Further in-depth immunological investigation is required to comprehend this intricate interaction of polymorphism in the current population.One of the study's limitations is that the data set is comparably smaller. It may be possible to address in a categorized way the functional significance of these polymorphisms in relation to immunological responses to tuberculosis.

6. Conclusion

The CCL2-2518 GG and -362 CC genotypes have been shown in this investigation to be significantly associated with tuberculosis. It has been observed that heterozygous CCL2-2518AG and -362GC are linked to resistance to PTB. It has been observed that the biallelic AC haplotype (CCL2-2518 A>G and -362 G>C) is susceptible to pulmonary tuberculosis. A complicated regulation system was proposed by the serum cytokine analysis.between IFN-γ concentration, IL-12p70, and CCL2/MCP-1. Positive correlations were found between CCL2 and IL-12, IFN-γ, and TNF-α. A normal CCL2 level is necessary for a normal Th1 response. In comparison to the A allele, the -2518G allele produces less CCL2, which causes an incorrect Th1 response and increases a host's susceptibility to tuberculosis.We attempted to unravel the function of the gene in the CCL2 promoter region in the current work, but more research is necessary to fully comprehend the intricate interplay between the polymorphisms in the CCL2 regulatory region.

Acknowledgments

We appreciate each and every participant's willingness to take part in this research. We appreciate the technical assistance provided by Mr. M.M. Alam, Mr. M. Stomar, Mr. Rajesh Rathore, and Mr. R Rawat. The Indian Council of Medical Research (ICMR) provided funding for Sanjay K. Biswas' Senior Research Fellowship under Grant No. 45/13/2010/CMB/BMS.

References

1. Global TB report, "20 th Edition, Executive summary2, 2015http://www.who.int/tb/publications/global_report/gtbr2015_executive_summry.pdf.
2. M. Möller and E. G. Hoal, "Current findings, challenges andnovel approaches in human genetic susceptibility to tuberculo-sis," Tuberculosis (Edinburgh, Scotland), vol. 90, no. 2, pp. 71–83, 2010.
3. M. Möller, E. D. Wit, and E. G. Hoal, "Past, present and futuredirections in human genetic susceptibility to tuberculosis,"FEMS Immunology & Medical Microbiology, vol. 58, no. 1,pp. 3–26, 2010.
4. B. J. Rollins, "Chemokines," Blood, vol. 90, no. 3, pp. 909–928,1997.
5. A. Zlotnik and O. Yoshie, "Chemokines: a new classificationsystem and their role in immunity," Immunity, vol. 12, no. 2,pp. 121–127, 2000.
6. A. Kipnis, R. J. Basaraba, I. M. Orme, and A. M. Cooper, "Roleof chemokine ligand 2 in the protective response to earlymurine pulmonary tuberculosis," Immunology, vol. 109,no. 4, pp. 547–551, 2003.
7. H. L. Collins and S. H. Kaufmann, "The many faces of hostresponses to tuberculosis," Imunology, vol. 103, no. 1, pp. 1–9, 2001.
8. H. M. Algood, J. Chan, and J. A. L. Flynn, "Chemokines andtuberculosis," Cytokine & Growth Factor Reviews, vol. 14,no. 6, pp. 467–477, 2003.
9. S. E. Jamieson, E. N. Miller, G. F. Black et al., "Evidence for acluster of genes on chromosome 17q11-q21 controlling sus-ceptibility to tuberculosis and leprosy in Brazilians," Genesand Immunity, vol. 5, no. 1, pp. 46–57, 2004.
10. P. O. Flores-Villanueva, J. Ruiz-Morales, C. H. Song et al., "Afunctional promoter polymorphism in monocyte chemoat-tractant protein is associated with increased susceptibility topulmonary tuberculosis," JEM 2005, vol. 12, pp. 1649–1658,2005.
11. T. Thye, S. Nejentsev, C. D. Intemann et al., "MCP-1 promotervariant −362C associated with protection from pulmonarytuberculosis in Ghana, West Africa," Human Molecular Genet-ics, vol. 18, no. 2, pp. 381–388, 2009.
12. M. Ganachari, J. A. Ruiz-Morales, J. C. Gomez de la Torre Pre-tell, J. Dinh, J. Granados, and P. O. Flores-Villanueva, "Jointeffect of MCP-1 genotype GG and MMP-1 genotype 2G/2Gincreases the likelihood of developing pulmonary tuberculosisin BCG-vaccinated individuals," PLoS one, vol. 5, no. 1,p. e8881, 2010
13. N. Arji, M. Busson, G. Iraqi et al., "The MCP-1 (CCL2) -2518GG genotype is associated with protection against pulmonarytuberculosis in Moroccan patients," Journal of Infection inDeveloping Countries, vol. 6, pp. 73–78, 2012.
14. T. Gong, M. Yang, L. Qi, M. Shen, and Y. du, "Association ofMCP-1 -2518A/G and -362G/C variants and tuberculosis sus-ceptibility: a meta-analysis," Infection, Genetics and Evolution,vol. 20, pp. 1–7, 2013.
15. G. Mishra, S. S. Poojary, P. Raj, and P. K. Tiwari, "Geneticpolymorphisms of _CCL2_ , _CCL5_ , _CCR2_ and _CCR5_genes in Sahariya tribe of North Central India: an associationstudy with pulmonary tuberculosis," Infection, Genetics andEvolution, vol. 12, no. 5, pp. 1120–1127, 2012.
16. B. Singh, J. Chitra, and P. Selvaraj, "CCL2, CCL3 and CCL4gene polymorphisms in pulmonary tuberculosis patients ofSouth India," International Journal of Immunogenetics,vol. 41, no. 2, pp. 98–104, 2014.
17. G. Tian, X. Li, H. Li, X. Wang, and B. Cheng, "Systematicmeta-analysis of the association between monocyte chemoat-tractant protein-1-2518A/G polymorphism and risk of tuber-culosis," Genetics and Molecular Research, vol. 14, no. 2,pp. 5501–5510, 2015.
18. S. F. Chu, C. M. Tam, H. S. Wong, K. M. Kam, Y. L. Lau, andA. K. S. Chiang, "Association between _RANTES_ functionalpolymorphisms and tuberculosis in Hong Kong Chinese,"Genes and Immunity, vol. 8, no. 6, pp. 475–479, 2007.
19. M. Möller, A. Nebel, R. Valentonyte, P. D. van Helden,S. Schreiber, and E. G. Hoal, "Investigation of chromosome17 candidate genes

in susceptibility to TB in a South Africanpopulation," Tuberculosis, vol. 89, no. 2, pp. 189–194, 2009.

20. Ethical guidelines for biomedical research on human partici-pants, "Indian Council of Medical Research New Delhi,"2006. http://icmr.nic.in/ethical_guidelines.pdf.
21. RNTCP, "Guidelines 2007-TBC India. Diagnosis of smearpositive pulmonary TB," http://www.tbcindia.org.[22] A. L. Vestal, "Identification test technique," in Procedure forIsolation and Identification of Mycobacteria. U.S. Departmentof Health, Education and Welfare Publication No. CDC-77-8230, Centres for Disease Control and Prevention, Atlanta,GA, 1977.
22. T. Vásquez-Loarte, M. Trubnykova, and H. Guio, "Geneticassociation meta-analysis: a new classification to assess ethnic-ity using the association of MCP-1-2518 polymorphism andtuberculosis susceptibility as a model," BMC Genetics,vol. 16, no. 1, p. 128, 2015.
23. W.-X. Feng, P. O. Flores-Villanueva, I. Mokrousov et al.,"CCL2−2518 (A/G) polymorphisms and tuberculosis suscepti-bility: a meta-analysis," The International Journal of Tubercu-losis and Lung Disease, vol. 16, pp. 150–156, 2012.
24. W.-X. Feng, I. Mokrousov, B.-B. Wang et al., "Tag SNP poly-morphism of CCL2 and its role in clinical tuberculosis inHan Chinese pediatric population," PLoS One, vol. 6, no. 2,p. e14652, 2011.
25. D. Nonghanphithak, W. Reechaipichitkul, W. Namwat,V. Lulitanond, V. Naranbhai, and K. Faksri, "Genetic poly-morphisms of CCL2 associated with susceptibility to latenttuberculous infection in Thailand," The International Journalof Tuberculosis and Lung Disease, vol. 20, no. 9, pp. 1242–1248, 2016.
26. K. Alagarasu, P. Selvaraj, S. Swaminathan, S. Raghavan,G. Narendran, and P. R. Narayanan, "CCR2, MCP-1, SDF-1a10 Journal of Immunology Research& DC-SIGN gene polymorphisms in HIV-1 infectedpatients with & without tuberculosis," The Indian Journalof Medical Research, vol. 130, no. 4, pp. 444–450, 2009.
27. D. R. Velez Edwards, A. Tacconelli, and C. Wejse et l, "MCP1SNPs and pulmonary tuberculosis in cohorts from WestAfrica, the USA and Argentina: lack of association or epistasiswith IL12B polymorphisms," PLoS One, vol. 7, no. 2,p. e32275, 2012.
28. P. Doherty and R. Zinkernagel, "Enhanced immunologicalsurveillance in mice heterozygous at the H-2 gene complex,"Nature, vol. 256, no. 5512, pp. 50–52, 1975.
29. E. Sinha, S. K. Biswas, M. Mittal et al., "Toll-like receptor 1 743A>G, 1805 T>G & Toll-like receptor 6 745 C>T gene poly-morphism and tuberculosis: a case control study of northIndian population from Agra (India)," Human Immunology,vol. 75, no. 8, pp. 880–886, 2014.
30. B. H. Rovin, L. Lu, and R. Saxena, "A novel polymorphism inthe MCP-1 gene regulatory region that influences MCP-1expression," Biochemical and Biophysical Research Communi-cations, vol. 259, no. 2, pp. 344–348, 1999.
31. M. W. Carr, S. J. Roth, E. Luther, S. S. Rose, and T. A. Springer,"Monocyte chemoattractant protein 1 acts as a T-lymphocytechemoattractant," Proceedings of the National Academy of Sci-ences, vol. 91, no. 9, pp. 3652–3656, 1994.
32. N. V. Serbina, T. Jia, T. M. Hohl, and E. G. Pamer, "Monocyte-mediated defense against microbial pathogens," AnnualReview of Immunology, vol. 26, no. 1, pp. 421–452, 2008.
33. C. D. Intemann, T. Thye, B. Förster et al., "MCP1 haplotypesassociated with protection from pulmonary tuberculosis,"BMC Genetics, vol. 12, no. 1, p. 34, 2011.