

# Lack of Association between rs1024611 (–2581 A/G) Polymorphism in CC-chemokine Ligand 2 and Susceptibility to Pulmonary Tuberculosis in Zahedan, Southeast Iran

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**Abstract:** Approximately 5–10% of subjects infected with *Mycobacterium tuberculosis* develop active tuberculosis. It has been proposed that genetic factors determine the host's vulnerability to tuberculosis. Chemokine (C-C motif) ligand 2 (CCL2), commonly known as monocyte chemoattractant protein-1 (MCP-1), plays a key role in protective immunity against *M. tuberculosis*. The present study was aimed to determine if there was an association between –2581 A/G single nucleotide polymorphism of CCL2 and pulmonary tuberculosis (PTB) in a sample of Iranian subjects. This case-control study was performed on 142 PTB and 166 healthy subjects. The polymorphism of CCL2 (rs1024611) was determined using tetra amplification refractory mutational system-polymerase chain reaction (tetra ARMS-PCR). There were no significant differences between PTB patients and control subjects regarding –2581 A/G single nucleotide polymorphism of CCL2. In conclusion, our results do not support an association of –2581 A/G polymorphism of CCL2 with PTB susceptibility.

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## Introduction

Tuberculosis (TB) still remains one of the important causes of mortality and mortality. The World Health Organization (WHO) estimates that there are more than 8 million new cases of tuberculosis (TB) annually, that about one third of the world's population is infected with TB (2 billion people) and that about 10% of these will progress to the disease state (Dye et al., 1999). Pulmonary tuberculosis (PTB) is still a major health problem in both industrialized and developing countries and it remains a leading infectious cause of death (Alavi-Naini et al., 2009; Naderi et al., 2009). It has been reported that genetic factors contribute considerably to the development of tuberculosis. Chemokine (C-C motif) ligand 2 (CCL2), commonly known as monocyte chemoattractant protein-1 (MCP-1), is a central component of the granulomatous response (Taub et al., 1995). Human MCP-1 is located on chromosome 17 (17q11.2), is composed of 76 amino acids and is 13 kDa in size (Van Coillie et al., 1999). It has been reported that a single nucleotide polymorphism in the CCL2 (–2518 A/G) influence the expression of this gene (Rovin et al., 1999). There are conflicting results regarding the association between –2581 A/G polymorphism of CCL2 and susceptibility to PTB (Jamieson et al., 2004; Flores-Villanueva et al., 2005; Chu et al., 2007; Buijtelts et al., 2008; Alagarasu et al., 2009; Ganachari et al., 2010). Therefore, the aim of the present study was to determine the possible association between –2581 A/G single nucleotide polymorphism of CCL2 and susceptibility to pulmonary tuberculosis in samples of Southeast Iranian patients.

## Material and Methods

### *Patients and control*

The study design and the enrolment procedure have been described previously (Naderi et al., 2009, 2011; Hashemi et al., 2011b). This case-control study was performed in the Research Center for Infectious Diseases and Tropical Medicine, Bou-Ali Hospital, Zahedan, Iran. 142 patients (54 male, 88 female; age = 50.1 ± 21.0 year) with confirmed PTB and 166 healthy subjects (82 male, 84 female; age = 50.7 ± 13.3 year) were included into the study.

The study was approved by the local ethics committee of Zahedan University of Medical Sciences and written informed consent was obtained from all subjects.

Genomic DNA was extracted from peripheral blood as described previously (Hashemi et al., 2010a) and stored at –20 °C. Amplification refractory mutation system polymerase chain reaction (ARMS-PCR) is a simple and rapid method for detection of single nucleotide polymorphism (Hashemi et al., 2010a, b, 2011a). We designed a tetra primer ARMS-PCR for the detection of –2581 A/G polymorphism of CCL2 (rs1024611). Two external primers (forward outer primer: 5'-TAACTGAGGATTCTGGACAG-3', reverse outer primer: 5'-TTATCTGATAAAGCCACAATC-3') and two inner primers (forward inner primer (A allele): 5'-GTGGGAGGCAGACAGATA-3', reverse inner primer

(G allele): 5'-AGAAAGTCTTCTGGAAAGTTAC-3') were used. Product sizes were; 255 bp for A allele and 379 bp for G allele, while the product size of the two outer primers was 594 bp as is shown schematically in Figure 1.

Reactions consisted of a total volume of 25 µl containing 250 µM dNTPs, 0.5 µM of each primer, 1.5 mM MgCl<sub>2</sub>, 1 U of Taq DNA polymerase (Roche Molecular Biochemicals, IN, USA), and ~50–100 ng of genomic DNA. Polymerase chain reaction cycling conditions were as follows: 5 min at 95 °C; 30 cycles of 30 s at 95 °C, 30 s at 59 °C and 40 s at 72 °C; 10 min at 72 °C (Corbett research, Australia). Each reaction was verified on a 2% agarose gel (Figure 2).

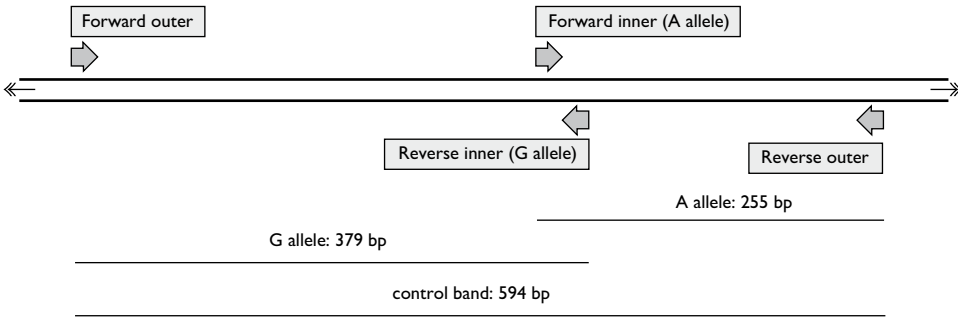


Figure 1 – Schematic diagram of the tetra ARMS assay for detection single nucleotide polymorphism of –2581 A/G in CCL2 gene. Two forward and two reverse primers are used to generate three potential PCR products. Primers FO and RO give a 594 bp product, which is used to control for DNA quality and quantity. Primers FI and RO amplify the A allele, generating a 255 bp product, and primers FO and RI generate a 379 bp product for the G allele.

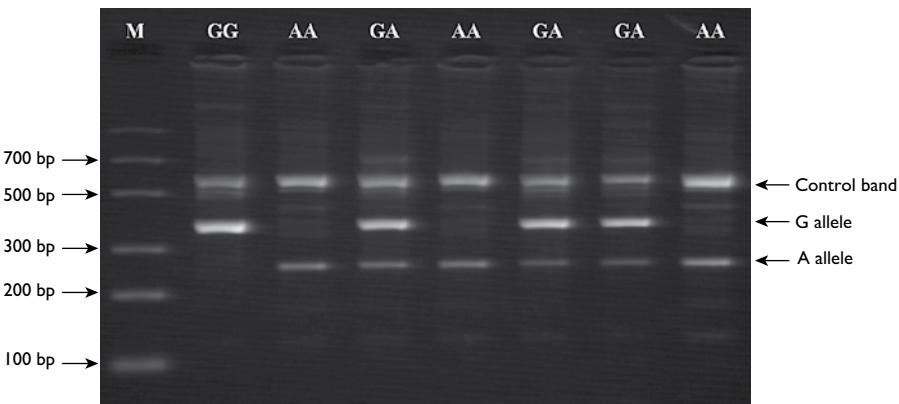


Figure 2 – Electrophoresis pattern of tetra amplification refractory mutation system-polymerase chain reaction (tetra ARMS-PCR) for detection of SNP in CCL2 –2581 A/G. M = DNA marker. Product sizes were 255 bp for A allele, 379 bp for G allele, and 594 bp for two outer primers (control band).

### Statistical analysis

Statistical analysis was performed using commercial software (SPSS for Windows, V 17, SPSS Inc., Chicago, IL, USA). Data were analyzed by chi-square. A P-value less than 0.05 were considered statistically significant.

### Results

The distribution of AG –2581 A/G polymorphism of CCL2 in PTB patients and normal subjects are shown in Table 1. The results showed that the frequency of homozygous AA, heterozygous AG, and homozygous GG in TB patients were 52.8%, 35.2%, and 12.0%, respectively. The frequency of AG –2581 polymorphism in healthy individuals was 50.0% for AA, 41.0% for AG, and 9.0% for GG. There was no significant differences in AG –2581 polymorphism of CCL2 among PTB and normal subjects ( $P=0.493$ ).

The distribution of allele's frequencies of –2581 CCL2 A/G in PTB and normal individuals was shown in Table 2. No significant differences was observed among PTB and control subjects regarding allele frequencies ( $P=0.987$ ). Our results showed that there were no statistically significant differences in distribution of genotype or allelic frequencies for the –2581 A/G polymorphism of CCL2 between controls and patients.

### Discussion

In this study, we found that the promoter polymorphism of CCL2 at position –2581 (rs1024611) relative to the ATG transcription start codon is not associated with susceptibility to PTB in a sample of Iranian patients.

Our results in accordance with the studies performed in the Brazilian multicase families (Jamieson et al., 2004), in a study group of Chinese TB patients from

**Table 1 – Genotypes frequencies of –2581 A/G polymorphism of CCL2 in pulmonary tuberculosis (PTB) and healthy subjects**

| Genotypes | PTB (n=142) | Healthy subjects (n=166) |
|-----------|-------------|--------------------------|
| AA        | 75 (52.8%)  | 83 (50.0%)               |
| AG        | 50 (35.2%)  | 68 (41.0%)               |
| GG        | 17 (12.0%)  | 15 (9.0%)                |

$\chi^2=1.41$ ,  $p=0.493$

**Table 2 – Distribution of allele frequencies of –2581 A/G polymorphism of CCL2 among pulmonary tuberculosis (PTB) and healthy subjects**

| Alleles | PTB (n=284) | Healthy subjects (n=332) |
|---------|-------------|--------------------------|
| A       | 200 (70.4%) | 234 (70.5%)              |
| G       | 84 (29.6%)  | 98 (29.5%)               |

$\chi^2=0.0003$ ,  $p=0.987$

Hong Kong (Chu et al., 2007) and Indian samples (Alagarasu et al., 2009). In contrast to our finding, it has been shown that CCL2 –2581 G allele is a risk factor for PTB (Flores-Villanueva et al., 2005; Buijtelts et al., 2008; Ganachari et al., 2010; Ben-Selma et al., 2011). While, Thye et al. have found opposite results. They found that CCL2 –2581 G allele is associated with resistance to PTB in the Ghanaian population and no effect at all on PTB susceptibility in the Russian sample (Thye et al., 2009). Recently, a meta-analysis which performed by Intemann et al. (2011) on the five pooled case-control studies did not show a significant association between CCL2 –2581 A/G polymorphism and susceptibility or protection to PTB.

The CCL2 –2581 G allele is known to increase the expression of the CCL2 gene and the subjects bearing the CCL2 genotype GG produce high concentrations of MCP-1, which inhibits production of IL-12p40 in response to *Mycobacterium tuberculosis* and increases the likelihood that *M. tuberculosis* infection will progress to active PTB (Flores-Villanueva et al., 2005). *In vitro* studies in human monocytes (Braun et al., 2000) and murine dendritic cells, demonstrating, that MCP-1 inhibits IL-12 production (Chensue et al., 1996; Omata et al., 2002).

The exact reasons as to why only a number of the individuals infected with *M. tuberculosis* develop clinical disease remains unknown. There are some evidences suggest that host genetic factors may be important risk factor for development of tuberculosis (Newport et al., 1996; Jouanguy et al., 1999; Guide and Holland, 2002; Hashemi et al., 2011b; Qu et al., 2011; Zheng et al., 2011).

The controversial reports from different parts of the world indicate that the impact of CCL2 –2581 A/G gene polymorphism on PTB susceptibility is probably influenced by ethnic origin.

In summary, this is the first report regarding the association between CCL2 –2581 A/G polymorphism and pulmonary tuberculosis in a sample of Iranian patients. Our results showed that there were no significant differences in distribution of genotype and allelic frequencies for the CCL2 –2581 A/G polymorphism between controls and PTB patients.

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