

The MCP-1 (CCL2) -2518 GG genotype is associated with protection against pulmonary tuberculosis in Moroccan patients

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Abstract

Introduction: Both monocyte chemoattractant protein-1 (MCP-1), also designated officially as chemokine (C-C motif) ligand 2 (CCL2), and interleukin-12 p40 (IL-12 p40) molecules, encoded by polymorphic genes, are central components of the immune response to infection by *Mycobacterium tuberculosis* (*Mtb*). Their genetic diversity has previously been associated with the outcome of tuberculosis (TB) infection. We investigated whether the MCP-1 -2518 A/G and the IL-12B (p40) +1188 A/C polymorphisms influence susceptibility to or resistance against pulmonary tuberculosis (PTB) in a Moroccan population group.

Methodology: Genomic DNA from 337 patients along with 204 healthy controls were genotyped for the above-mentioned genetic variations using polymerase chain reaction-based restriction fragment length polymorphism assay.

Results: We found a higher prevalence of homozygous MCP-1 -2518 G allele in healthy individuals than in patients ($p = 0.04$; odds ratio = 0.35; 95% confidence interval = 0.13 - 0.86), suggesting a potential protective effect, whereas analysis of IL-12B +1188 variation failed to reveal any such association.

Conclusion: Our results are in agreement with recent findings in Ghanaian patients, complying with the known genetic admixture of the Moroccan population.

Key words: pulmonary tuberculosis; monocyte chemoattractant protein-1; interleukin-12 p40; polymorphism; Morocco

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Introduction

Tuberculosis (TB) is a major cause of microbial-related morbidity and mortality worldwide among patients developing active disease [1]. In Morocco, the incidence has been estimated to be 81 per 100,000 inhabitants, affecting mostly young adults with the consequent negative socio-economic impact [2]. Nevertheless, only 10% of individuals infected by the causative pathogen, *Mycobacterium tuberculosis* (*Mtb*), develop active disease [1]. Complex interactions between host genetic diversity, pathogen characteristics, and other environmental factors seem to contribute to such differential disease susceptibility [3,4]. Both monozygotic/dizygotic twin studies [5,6] as well as ethnic differences in infection rate support this

hypothesis [7]. Identification of predictive markers for disease risk, especially those related to immune-mediated responses, has become the major focus of several studies. It has been shown that macrophage/*Mtb* interactions induce the production of a number of chemokines. Among them, the monocyte chemoattractant protein-1 (MCP-1), also designated officially as chemokine (C-C motif) ligand 2 (CCL2), seems to be a key component of tuberculous granuloma constitution with consequent pathogen containment through Th1 pathway polarization and triggering [8-10]. In this context, interleukin-12 (IL-12), a heterodimeric Th1 cytokine composed of two subunits, (p35 and p40), encoded by two distinct genes (*IL-12A* and *IL-12B*), is also pivotal in defense against

intracellular pathogens such as *Mtb* [11]. In animal models, the p40 subunit, a component of both IL-12 and IL-23 cytokines, was shown to be important for protection against mycobacterial infection [12].

Both *MCP-1* and *IL-12B* genes exhibit genetic polymorphisms and several single nucleotide polymorphisms (SNP) have been reported. Some of these variations have been associated with TB outcomes, albeit the status of association in terms of susceptibility/resistance seems to vary depending upon the studied population. A functional A to G polymorphism at position -2518 of the MCP-1 promoter region, known to upregulate MCP-1 expression [13], had been associated with increased susceptibility to pulmonary tuberculosis (PTB) in Mexican, Korean and Peruvian patients [14,15] but not in other population groups of different ethnicities [16-18]. Moreover, a recent study involving a very large cohort of patients as well as nuclear families from Ghana, Africa, revealed a strong association between the high expressor MCP-1 -2518 G allele and resistance to TB [18]. Similarly, a number of functionally relevant SNPs in the IL-12B gene, including the promoter [19] and the 3' untranslated region (UTR) [20,21], have been shown to influence the IL12B mRNA [22] and IL-12p70 protein expression [19,23]. Among them, an A to C change at position +1188 in the 3'UTR region has been related to diminished IL-12 cytokine production [22] and implicated in different pathological settings including mycobacterial infections such as TB [24,25].

Given the demonstrated role of both MCP-1 and IL-12 molecules in antimycobacterial defense on the one hand, and the yet to be understood potential interactions between them in conferring protection against/susceptibility to TB on the other hand, we investigated concomitantly the potential influence of the *MCP-1* -2518 A/G and the *IL-12B* +1188 A/C polymorphisms on PTB genetic susceptibility in a retrospective case-control study of Moroccan individuals.

Methodology

The present study included 337 newly diagnosed patients with PTB attending the Moulay-Youssef Hospital (Rabat, Morocco), which covers a large geographical area of the country in enrolling such patients. The diagnosis was based on sputum positive microscopy, confirmed by positive *Mtb* culture tests. Any case with the absence of any previous history of tuberculosis along with positive clinical and chest

radiology findings was diagnosed as a first episode of the disease. All patients were negative for HIV, hepatitis B/C, and had no antecedents of immune-mediated disorders. The mean age at diagnosis was 38 ± 16.68 years (mean \pm SD) [range, 18 to 86 years] and the male-to-female ratio was 1.3 males for every female (195 males/142 females). The control group consisted of 204 healthy blood donors free of history of TB or immune-related diseases and was recruited at the blood bank center of Rabat. The male-to-female ratio and the mean age of this cohort were respectively 1.2 males for every female (113 males/91 females) and 30 ± 15.75 years (mean \pm SD) [range, 18 to 57 years].

All cases and controls were from either Arab or Berber population groups (or issued from both ethnicities) and shared globally similar socio-economic conditions and lifestyle. The medical school ethics committee of Mohamed V University of Rabat approved the study and informed consent was obtained from patients and controls.

From all samples, DNA was extracted from peripheral blood leukocytes using a standard salting-out procedure. Genotypes of both *MCP-1*-2518 A/G (rs1024611) and *IL-12B* +1188 A/C (rs3212227) were characterized by a polymerase chain reaction-based restriction fragment length polymorphism as previously described [14,21].

Comparisons of genotype and allele frequencies between patients and controls were performed using the Chi-square test with Yates's correction or Fisher exact test wherever appropriate. *P* values (two tailed) were corrected (*pc*) using the Bonferroni method and findings considered statistically significant for *pc* equal to or less than 0.05. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated to assess the relative risk conferred by a specific allele or genotype. Moreover, a logistic regression including sex and age as covariates was also performed. Deviation from the Hardy-Weinberg equilibrium was analyzed using χ^2 testing.

Results

For both SNPs, the observed genotype distribution among controls and cases satisfied the expected Hardy Weinberg ratio.

We found that the *MCP-1* -2518 G allele was more frequent in controls than in patients, but failed to reach statistical significance after correction for the number of comparisons (27% versus 22% in controls and patients respectively, *pc* = 0.09; unadjusted OR = 0.75, 95% CI = 0.56 – 1.01) (Table 1). Nevertheless,

Table 1. MCP-1 -2518 allele and genotype frequencies among patients and healthy controls

MCP-1 -2518 A/G	Patients (n = 337)		Controls (n = 204)		p	pc	OR, 95 %CI
	Allele	n	f	n			
-2518 A	528/674	78%	298/408	73%	–	–	1.33 (0.99-1.79)
-2518 G	146/674	22%	110/408	27%	0.047	0.09 (NS)	0.75 (0.56-1.01)
Genotype	n	f	n	f	p	pc	OR 95 %CI
-2518 A/A	200/337	59%	109/204	54%	0.178	NS	1.27 (0.88-1.83)
-2518 A/G	128/337	38%	80/204	39%	0.775	NS	0.95 (0.65-1.83)
-2518 G/G	09/337	03%	15/204	07%	0.016	0.04	0.35 (0.13-0.86)

n: number OR: odds ratio f: frequency CI: confidence interval pc: corrected P-value NS: non-significant

analysis of the MCP-1 genotype distribution revealed that the homozygous state of the MCP-1 -2518 G allele was significantly more prevalent in healthy controls than in TB patients (7% versus 3%, $p_c = 0.04$; OR = 0.35, 95% CI = 0.13 - 0.86) (Table 1). In addition, the observed association between the GG genotype and protection against PTB was further confirmed by a logistic regression analysis ($p = 0.016$, adjusted OR = 0.33, 95%CI = 0.13 - 0.81) (Table 1).

Concerning the IL-12+1188 A/C polymorphism, no significant difference was noted in the distribution of allele and genotype frequencies between both groups (allele frequencies: A: 67% versus 70%, C: 33% vs 30% and genotype frequencies: AA: 45% vs 51%, AC: 44% vs 38% and CC: 11% vs 11% among patients and controls respectively). Finally, statistical cross-analysis of the two studied polymorphisms among patients and controls excluded genetic interactions between them with respect to disease susceptibility (data not shown).

Discussion

Potent immune responses against tuberculosis infection require adequate production of major chemokines and cytokines such as MCP-1 and IL-12, the expression of which is in part controlled by the genetic diversity of their encoding genes. In line with it, the MCP-1 -2518 A/G polymorphism had previously been associated with PTB in Mexican and Korean patients, two genetically distinct population groups. Indeed, it was shown that the MCP-1 -2518 G allele was associated with the disease in a dose-

dependant manner (odds ratios for GG genotypes were more than two-fold higher than those for AG genotypes) and was functionally related to high serum levels of the MCP-1 molecule with a concomitant decrease in IL-12 p40 both in patient sera and in cultured monocytes after stimulation with *Mtb* antigens [14]. Considering these findings, the authors hypothesized that the MCP-1 -2518 G allele-driven overproduction of MCP-1 down-regulates IL-12 p40 cytokine production with consequent escape of *Mtb* infection from Th1 response. In a much more recent work involving another Mexican population group as well as Peruvian individuals, the same team confirmed the association of the MCP-1 -2518 G allele and GG genotype with the active form of the disease but without the above-mentioned dose effect [15].

However, other genetic studies involving patient cohorts from various population groups including Brazilians, Chinese and Russians, did not confirm the above findings [16-18], and may suggest a population-specific influence of the studied polymorphism. This may imply that the MCP-1 variant linked polymorphisms may differ among population groups and may be responsible for the elevated MCP-1 levels predisposing to PTB. Neither can we exclude the possibility that the environmental variance such as differences in *Mtb* strains in different study populations could influence the effect of genetic variation. Of note, the higher prevalence of the MCP-1 -2518 GG genotype in controls as compared to PTB patients in our study suggests that

Table 2. *MCP-1* -2518 genotype and allele frequency comparison between Moroccan and Ghanaian populations

MCP-1 -2518 A/G	Morocco, North		Ghana, West Africa	
	Patients (n = 337)	Controls (n = 204)	Patients (n = 1964)	Controls (n = 2312)
Allele	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)
-2518 A	0.78	0.73	0.83	0.80
-2518 G	0.22	0.27	0.17	0.20
Genotype	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)
-2518 A/A	0.59	0.54	0.69	0.64
-2518 A/G	0.38	0.39	0.28	0.32
-2518 G/G	0.03	0.07	0.03	0.04
References	Our study		Thye T <i>et al.</i> (2009) [18]	

the protection conferred by the *MCP-1* -2518 G allele could be recessive.

This differs somewhat from a large survey of the Ghanaian population where both the *MCP-1* -2518 G allele and the AG genotype occurred with higher prevalence among controls than cases [18] (Table 2). The difference could be due to a higher consanguinity rate in Moroccans than in Ghanaians, inflating the homozygosity for the GG genotype in the former. Alternatively, the linked polymorphisms that might be directly involved in protection may differ between these two populations. Indeed, extended analysis of the genetic diversity of the Ghana cohort revealed that a G to C polymorphism at *MCP-1* -362 position correlated better with resistance to PTB than the *MCP-1* -2518 variation, strongly suggesting that the initial finding of its association with PTB likely reflects the linkage disequilibrium (LD) between these SNPs. Using logistic regression analysis, Thye *et al.* demonstrated further that the protection was exclusively conferred by the *MCP-1* -362 C variant. In addition, in the same study, the analysis of the *MCP-1* -2518 polymorphism distribution in a large sample of non-African patients (*i.e.*, Russians) failed to reveal any such association. Because the LD patterns can vary among population groups, studies based on *MCP-1* -2518 polymorphism may generate conflicting association data.

A similar association between the *MCP-1* -2518 promoter variant and resistance to PTB and a similar trend in the allele and genotype distribution (Table 2) observed both in Moroccan and Ghanaian populations may reflect the known genetic admixture between

these two African populations. Indeed, by its strategic location in Africa, Morocco was a crossroads of trans-Saharan caravans. In addition, the capital of the empire of Ghana was conquered by the Moroccan empire of Almoravids in 1076 [26]. Furthermore, recorded evidence concerning the transfer of black populations to Morocco from the major markets of Sudan, Djenné or Timbuktu (the main destinations of the caravans from the north of Ghana) and the inter-population marriages for both economic and military reasons during the period from 1672 to 1727 period [27] additionally ensured strong admixture between the people of Morocco and populations of sub-Saharan Africa.

Although the *IL-12B* +1188 polymorphism is functionally relevant [22, 23] in various immune-related disorders including mycobacterial infections [24, 25], and other genes belonging to the IL-12/IL-23/interferon gamma axis have previously been reported to be associated with PTB in Moroccan patients [28], we failed to reveal an association with PTB susceptibility. Nevertheless, our data are in agreement with previous findings involving patients with PTB in case/control and family-based transmission disequilibrium test settings [29, 30]. The studies involving Indians or Hong Kong Chinese patients showing association of PTB susceptibility with specific *IL-12B* haplotypes [24, 31], suggest that the implication of *IL-12B* genetic diversity in PTB susceptibility may be through an additive effect of several polymorphisms.

The limitations of our study include the lack of data on *MCP-1* -362 polymorphism and its LD status with

MCP-1 -2518 polymorphism. Lack of a sufficient amount of available DNA prevented us from analyzing the *MCP-1 -362* polymorphism for our study population and a new sample collection is in our agenda. In addition, we intend to extend and explore the potential implication of the *IL-12B* locus by studying its haplotype structure in our Moroccan patient and control populations.

In summary, the present study, albeit contrasting with some previous reports, is in agreement with the recent study in Ghanaians where the *MCP-1 -2518 G* allele was associated with resistance to PTB and can be explained by the known genetic admixture between these two African population groups. In the future, a much larger study to confirm the lack of association between *IL-12B +1188* and susceptibility/resistance to PTB needs to be performed. Additionally, causal variant(s) that could be in LD with *MCP-1 -2518* must be identified and *Mtb* subtype characterization needs to be performed to gain further insight into PTB susceptibility in Morocco.

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