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MCP-1 promoter variant −362C associated with protection from pulmonary tuberculosis in Ghana, West Africa

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Current endeavour focuses on human genetic factors that contribute to susceptibility to or protection from tuberculosis (TB). Monocytes are crucial in containing *Mycobacterium tuberculosis* infection, and the monocyte chemoattractant protein-1 (MCP-1) cytokine plays a role in their recruitment to the site of infection. The G allele of the *MCP-1* promoter polymorphism at position −2581 relative to the ATG transcription start codon has been described to be associated in Mexican and Korean TB patients with increased susceptibility to TB. We genotyped this and additional *MCP-1* variants in sample collections comprising more than 2000 cases with pulmonary TB and more than 2300 healthy controls and 332 affected nuclear families from Ghana, West Africa, and more than 1400 TB patients and more than 1500 controls from Russia. In striking contrast to previous reports, *MCP-1* −2581G was significantly associated with resistance to TB in cases versus controls [odds ratio (OR) 0.81, corrected *P*-value (P_{corr}) = 0.0012] and nuclear families (OR 0.72, P_{corr} = 0.04) and not with disease susceptibility, whereas in the Russian sample no evidence of association was found (*P* = 0.86). Our and other results do not support an association of *MCP-1* −2581 with TB. In the Ghanaian population, eight additional *MCP-1* polymorphisms were genotyped. *MCP-1* −362C was associated with resistance to TB in the case–control collection (OR 0.83, P_{corr} = 0.00017) and in the affected families (OR 0.7, P_{corr} = 0.004). Linkage disequilibrium (LD) and logistic regression analyses indicate that, in Ghanaians, the effect results exclusively from the *MCP-1* −362 variant, whereas the effect of −2581 may in part be explained by its LD with −362.

INTRODUCTION

Increasing evidence indicates that susceptibility to tuberculosis (TB) in humans largely depends on the individual genetic architecture (1). Among the genetic variants implicated in studies to determine their impact on TB susceptibility, no major risk factor has been identified so far. Observed differences in genotype frequencies between TB cases and controls were often small and have resulted in either weak associations or failure of confirmation in other studies and different ethnicities.

Linkage of TB susceptibility with the 17q chromosomal region (2,3) where monocyte chemoattractant protein-1

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(*MCP-1*) clusters together with *CCL7*, *CCL11*, *NOS2A*, *CCL3-5* and *CCR7* has previously been observed. It was also reported that a promoter variant of the gene encoding *MCP-1* [syn. *CCL2* (MIM +158105; 17q11.2–q12)] is associated with increased susceptibility to clinical TB in Mexicans and Koreans (4). The risk to develop pulmonary TB of both Mexican and Korean carriers of the *MCP-1* allele G at position −2581 relative to the ATG transcription start codon (rs1024611, alias −2518; http://snpper.chip.org/bio/view-snpset/moao587463/T) was significantly higher compared with individuals carrying the A allele. However, no association of *MCP-1* −2581G has been found in the Brazilian multicase families (3) and in a study group of Chinese TB patients from Hong Kong (5).

MCP-1 belongs to the CC chemokines (two adjacent cysteine residues close to the amino terminus) which trigger chemokine receptors on monocytes and other immune cells. The receptor for MCP-1 is the chemokine, CC motif, receptor 2 (monocyte chemotactic protein 1 receptor; CCR2A), a receptor that serves in its isomeric form (CCR2B) in the recognition of both MCP-1 and MCP-3. Different cell types are able to produce MCP-1, whereby it is not clear whether MCP-1 is mainly secreted by infected cells or by uninfected cells in response to inflammatory cytokines or to microbial components (6). Expression of MCP-1 may be found in many disorders characterized by mononuclear cell infiltration. Upon infection with *Mycobacterium tuberculosis*, MCP-1 is preferentially produced by CD14+ blood monocytes, but also by A549 alveolar epithelial cells (2,7). Microbial components are able to stimulate secretion of cytokines and expression of the CCR2 receptor and to induce, dependent on the presence of MCP-1, migration of monocytes to the site of infection.

The mode of action of MCP-1 in migration and attraction of monocytes is not completely understood. MCP-1 could be released directly from infected cells and, by forming a chemical gradient, escort sensitive monocytes to the site of tissue injury. High and balanced levels of serum MCP-1 could, however, abrogate effective recruitment of monocytes to sites of lesions by depletion of the gradient. An alternative scenario is that chemokines bind to distinct glycosaminoglycans in the bone marrow and pilot monocytes from there via the bloodstream to affected tissues (6). Recent results from studies of genetically modified mice have demonstrated effects of both MCP-1 and CCR2 on the development of T helper cells, with MCP-1 preferably stimulating Th2 immune responses and CCR2 rather triggering Th1 polarization (8).

Several findings, although partly contradictory and possibly dependent on the ethnic background of the population studied, as well as the biological function of MCP-1, strongly suggest an influence of *MCP-1* variation on TB susceptibility. Positive linkage of the 17q region (2) and an association of *MCP*-1 −2581G (rs1024611) with TB susceptibility in Mexican and Korean TB patients (4) have been observed. In contrast, no association of *MCP*-1 −2581 was found in Brazilian multicase families (3) and in a Chinese patient group from Hong Kong (5).

We have studied the *MCP-1* −2581 and eight additional *MCP-1* polymorphisms in a sample of more than 2000 sputum/culture-positive pulmonary TB patients and more than 2300 healthy control individuals from Ghana and also assessed the role of *MCP-1* −2581 variation in a TB case–control collection (more than 1400 TB patients and more than 1500 controls) from Russia.

RESULTS

Ghanaian study population

A total of nine *MCP-1* variants were genotyped in 2010 Ghanaian pulmonary TB cases and 2346 healthy controls. Assuming an approximative TB prevalence of 0.003 in West Africa, a frequency of 0.1 for high-risk alleles and a genotype relative risk of 1.2 ($\alpha = 0.05$), a detection power of >80% was achieved for additive and multiplicative models with the Ghanaian sample. The frequencies of the nine *MCP-1* variants were adjusted for gender, age and ethnicity. Genotype frequencies did not deviate from Hardy–Weinberg equilibria (HWE) beyond that expected at random ($P < 0.01$).

Alleles and genotypes

Allele and genotype frequencies of the *MCP-1* variants tested in the Ghanaian sample are summarized in Tables 1 and 2 and the respective r^2 values of pairwise linkage disequilibria (LDs) are given in Figure 1.

Allele frequencies of SNPs at positions −11822, −2581, −362 and +5356 differed significantly between cases and controls with corrected *P*-values (P_{corr}) of 1.0×10^{-3}, 1.2×10^{-3}, 1.7×10^{-4} and 9.3×10^{-4}, respectively (Table 1). In particular, the promoter allele −2581G, which has previously been described to be associated with susceptibility to TB (4), was significantly more frequent among control individuals [17% cases versus 20% controls; odds ratio (OR) 0.81, confidence interval (CI) 0.73–0.91, nominal *P*-value (P_{nom}) = 2×10^{-4}, $P_{corr} = 1.2 \times 10^{-3}$]. The promoter variant at position −362C, which was in moderate linkage with −2581G ($r^2 = 0.27$), was more strongly associated with protection from TB than was −2581G. With its occurrence of 42% in cases and 47% in controls, an OR of 0.83 (CI 0.76–0.90; $P_{nom} = 1.9 \times 10^{-5}$, $P_{corr} = 1.7 \times 10^{-4}$) was achieved. Two further variants, −11822 and +5356, were identified after imputation. Genotyping of these two variants, which were in linkage of $r^2 = 0.72$ and $r^2 = 0{,}84$, respectively, with −362 revealed similar associations of −11822A and +5356T with resistance to TB.

Genotype frequencies were calculated in trend tests to compare them in an additive model (Table 2). The strongest association was found for the C variant at position −362 which occurred significantly more frequently among controls than in cases [OR (trend) 0.83, CI 0.76–0.91, $P_{corr} = 2.3 \times 10^{-4}$), followed by the T variant at position +5356 associated with resistance (OR 0.85, CI 0.78–0.92, $P_{corr} = 1.1 \times 10^{-3}$). *MCP-1* −11822A was more frequent among controls (OR 0.85, CI 0.77–0.92, $P_{corr} = 1.2 \times 10^{-3}$), and the A variant at position −2581 was more frequent in cases (OR 0.81, CI 0.73–0.91, $P_{corr} = 1.8 \times 10^{-3}$). The differences that we observed were not dependent on differing mycobacterial species or genotypes or the age of patients, as, when stratifying

Table 1. Allelic associations

MCP-1 allele	Cases (*n*) (frequency)	Controls (*n*) (frequency)	OR 95% CI	P_{nom}	P_{corr}
Ghana					
−11822G	2537 (0.63)	2778 (0.59)	1		
−11822A	1467 (0.37)	1898 (0.41)	0.84 (0.77–0.92)	**0.00013**	**0.001**
−2581A	3256 (0.83)	3692 (0.80)	1		
−2581G	672 (0.17)	932 (0.20)	0.81 (0.73–0.91)	**0.0002**	**0.0012**
−2138A	3759 (0.96)	4408 (0.96)	1		
−2138T	177 (0.04)	192 (0.04)	1.09 (0.89–1.35)	0.41	
−2134T	3796 (0.96)	4478 (0.97)	1		
−2134G	140 (0.04)	122 (0.03)	1.35 (1.05–1.73)	**0.017**	
−1549A[a]	3170 (0.80)	3600 (0.79)	1		
−1549T	780 (0.20)	980 (0.21)	0.89 (0.80–0.99)	**0.038**	
−362G	2266 (0.58)	2441 (0.53)	1		
−362C	1670 (0.42)	2161 (0.47)	0.83 (0.76–0.90)	**0.000019**	**0.00017**
+900C	2906 (0.74)	3496 (0.76)	1		
+900T	1034 (0.26)	1122 (0.24)	1.12 (1.01–1.24)	**0.025**	
+3318C[a]	3178 (0.80)	3621 (0.78)	1		
+3318T	784 (0.20)	1003 (0.22)	0.89 (0.80–0.98)	**0.024**	
+5356C	2398 (0.61)	2634 (0.57)	1		
+5356T	1556 (0.39)	2014 (0.43)	0.84 (0.77–0.92)	**0.00013**	**0.00093**
Russia					
−2581A	2048 (0.71)	2168 (0.71)	1		
−2581G	832 (0.29)	890 (0.29)	0.99 (0.89–1.11)	0.86	

P-values are adjusted for age, gender and ethnicity. P_{nom}, nominal *P*-value; P_{corr}, *P*-value after Bonferroni-Holm correction.
[a]Variants −1549 and +3318 are in almost perfect LD ($r^2 = 0.98$).

Table 2. Genotype associations

MCP-1 GT	Cases (*n*) (frequency)	Controls (*n*) (frequency)	OR 95% CI	P_{nom}	P_{corr}	OR (trend) 95% CI	P_{nom}	P_{corr}
Ghana								
−11822GG	817 (0.41)	827 (0.35)	1			0.85 (0.77–0.92)	**0.00015**	**0.0012**
−11822AG	903 (0.45)	1124 (0.48)	0.81 (0.71–0.93)	**0.002**	**0.014**			
−11822AA	282 (0.14)	387 (0.17)	0.73 (0.61–0.88)	**0.001**	**0.007**			
−2581AA	1355 (0.69)	1472 (0.64)	1			0.81 (0.73–0.91)	**0.0003**	**0.0018**
−2581AG	546 (0.28)	748 (0.32)	0.79 (0.69–0.90)	**0.001**	**0.006**			
−2581GG	63 (0.03)	92 (0.04)	0.73 (0.53–1.02)	0.064				
−2138AA	1796 (0.91)	2113 (0.92)	1			1.09 (0.89–1.35)	0.4	
−2138AT	167 (0.08)	182 (0.08)	1.09 (0.87–1.36)	0.44				
−2138TT	5 (<0.01)	5 (<0.01)	1.27 (0.36–4.41)	0.71				
−2134TT	1835 (0.93)	2178 (0.95)	1			1.34 (1.05–1.72)	**0.019**	
−2134TG	126 (0.06)	122 (0.05)	1.22 (0.94–1.58)	0.13				
−2134GG	7 (<0.01)	0	NA					
−1549AA	1286 (0.65)	1410 (0.61)	1			0.89 (0.81–0.99)	**0.039**	
−1549AT	598 (0.30)	780 (0.34)	0.83 (0.73–0.95)	**0.006**	**0.024**			
−1549TT	91 (0.05)	103 (0.04)	0.97 (0.72–1.30)	0.84				
−362GG	672 (0.34)	654 (0.28)	1			0.83 (0.76–0.91)	**0.000026**	**0.00023**
−362CG	922 (0.47)	1133 (0.49)	0.80 (0.69–0.92)	**0.001**	**0.009**			
−362CC	374 (0.19)	514 (0.22)	0.70 (0.59–0.83)	**0.00005**	**0.00045**			
+900CC	1069 (0.54)	1326 (0.57)	1			1.12 (1.01–1.24)	0.025	
+900CT	768 (0.39)	844 (0.37)	1.14 (1.00–1.29)	**0.045**				
+900TT	133 (0.07)	139 (0.06)	1.21 (0.94–1.56)	0.14				
+3318CC	1286 (0.65)	1412 (0.61)	1			0.89 (0.80–0.98)	0.024	
+3318CT	606 (0.31)	797 (0.34)	0.83 (0.72–0.94)	**0.004**	**0.02**			
+3318TT	89 (0.04)	103 (0.04)	0.95 (0.71–1.28)	0.74				
+5356CC	746 (0.38)	747 (0.32)	1			0.85 (0.78–0.92)	**0.00016**	**0.0011**
+5356CT	906 (0.46)	1140 (0.49)	0.80 (0.70–0.92)	**0.001**	**0.008**			
+5356TT	325 (0.16)	437 (0.19)	0.73 (0.61–0.88)	**0.001**	**0.008**			
Russia								
−2581AA	726 (0.50)	794 (0.52)	1			0.99 (0.89–1.11)	0.86	
−2581AG	596 (0.41)	580 (0.38)	1.12 (0.97–1.31)	0.13				
−2581GG	118 (0.08)	155 (0.10)	0.83 (0.64–1.08)	0.17				

GT, genotype; OR (trend), estimates of an additive genetic model.
P-values are adjusted for age, gender and ethnicity. P_{nom}, nominal *P*-value; P_{corr}, *P*-value after Bonferroni–Holm correction.
[a]Variants −1549 and +3318 are in almost perfect LD ($r^2 = 0.98$; Fig. 1).

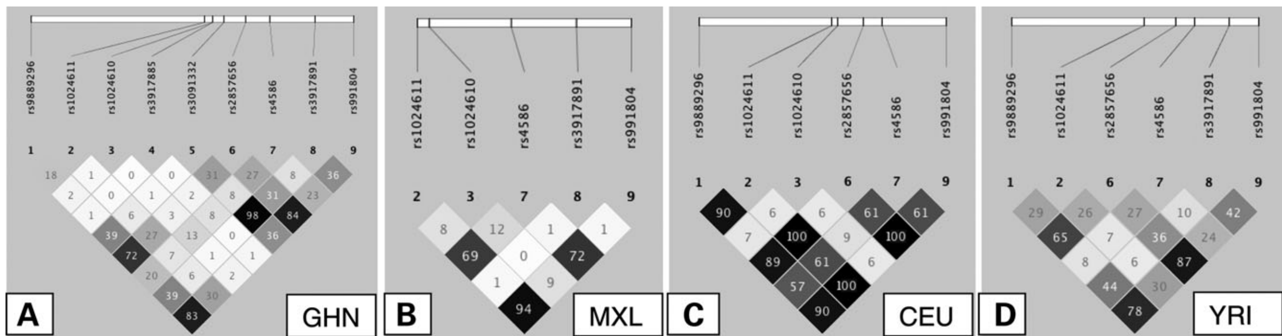

Figure 1. Pairwise LD (r^2) plots of *MCP-1* variants of the Ghanaian study group (GHN) (**A**), a Mexican population from California (MXL) (**B**), a population of the US residents of northern and western European ancestry (CEU) (**C**), the Yoruba population from Nigeria (YRI) (**D**). MXL, CEU and YRI data are extracted from the HapMap database. Nucleotide positions of rs numbers are given in Table 6. LDs compared between the different populations consisted of the same allelic combinations.

cases for the mycobacterial species *M. tuberculosis* and *Mycobacterium africanum* or grouping cases and controls according to age (6–30, >30 years), respectively, the findings remained significant (data not shown).

Haplotypes

Haplotypes and LD were reconstructed with the UNPHASED and HaploView software, respectively (Table 3, Fig. 1). The global and adjusted (age, gender, ethnicity) *P*-value of haplotype comparisons (P_g) was 2.6×10^{-5}. The *P*-value after permutation analyses to correct for multiple testing (10 000 permutations) was 1×10^{-4}. *MCP-1* −2581G/−362C was strongly associated with resistance to TB. Inclusion in haplotype reconstruction of the two other variants that we found associated with protection, −11822A and +5356T (Tables 1 and 2), did not affect the association ($P_g = 2.4 \times 10^{-4}$).

Interaction analysis of MCP-1 −2581 and −362

We tested whether the effect of *MCP-1* −362 was independent of that of *MCP-1* −2581 in a logistic regression model. A likelihood ratio (LR) test was applied to evaluate whether a given variant improved the models as given in Table 4. Focusing on the main effects of the −362 and −2581 variants in model 3 did not refine the predictive value of the phenotype than did model 2 with the −362 variant only (model 3 versus model 2; $P = 0.15$). Thus, the −362 variant was verified to exclusively explain the observed association with resistance to TB, however, implying that the association of the −2581 variant was not independent of that of *MCP-1* −362. To analyse synergistic effects between the −362 and −2581 variants, interaction terms were tested in logistic regression calculations. When comparing model 4 (interaction terms of −2581 and −362) with model 3 (individual variants), no improvement in the potential to predict the disease status was observed ($P = 0.16$).

To test whether the further variants that gave significant associations, *MCP-1* −11822 and +5356, were associated with the TB phenotype independently of *MCP-1* −362, each variant was added in turn to the regression model. None of these variants improved the model that predicts the phenotype (data not shown).

Transmission disequilibrium test

The results of the transmission disequilibrium tests (TDT; *MCP-1* −2581 and *MCP-1* –362) are given in Table 5. The TDT is robust against biases caused by ethnic stratification. TDT revealed less than expected at random transmission of the −362G allele to TB patients (OR 0.7, $P = 0.003$), indicating the protective effect of this variant and supporting our finding in the Ghanaian case–control sample. Permutation analyses (10 000 permutations) to correct for multiple testing confirmed the results with $P_{corr} = 0.04$ (*MCP-1* −2581) and $P_{corr} = 0.004$ (*MCP-1* −362).

Russian study population

The *MCP-1* −2581 (rs1024611) polymorphism was genotyped in 1440 TB patients and 1529 controls from Russia. Given that we observed a minor allele frequency of 0.29, this sample provided 90% power to detect an allele OR of 1.2 at $P = 0.05$, assuming a multiplicative model. We did not find an association with TB susceptibility (OR 0.99, $P = 0.86$). Since HapMap CEU data suggested that the *MCP-1* −2581 and −362 SNPs are in perfect LD in populations of European descent ($r^2 = 1.0$, Fig. 1), we did not genotype the *MCP-1* −362 variant in the Russian study group.

Meta-analysis of five study populations

The meta-analysis of the association between *MCP-1* −2581A>G variant and susceptibility to TB in five case–control studies of five ethnicities (Ghanaians, Russians, Chinese, Mexicans, Koreans) showed differences in associations between studies as indicated by a significant test for heterogeneity ($P < 0.001$). As heterogeneity occurred, a random effect model had to be applied. The DerSimonian and Laird algorithm showed that *MCP-1* G was not significantly associated with susceptibility to TB (pooled OR 1.39, CI 0.95–2.03, $P = 0.09$).

Table 3. Haplotype associations

Haplotype −2581	−362	Cases (*n*) (frequency)	Controls (*n*) (frequency)	OR 95% CI	*P*-value
A	G	2214 (0.58)	2405 (0.53)	1.21 (1.11–1.32)	**0.000014**
A	C	976 (0.25)	1227 (0.27)	0.92 (0.83–1.01)	0.088
G	C	658 (0.17)	906 (0.20)	0.82 (0.74–0.92)	**0.0003**

ORs and *P*-values refer to comparisons of one haplotype to the others combined.

Table 4. Model comparisons of main effects including *MCP1* −2581 and −362

Model	Model terms	Model comparisons	LR test, *P*-value
0			
1	MCP1 −2581	1 versus 0	**0.0006**
2	MCP1 −362	2 versus 0	**0.00003**
3	MCP1 −2581 + MCP1 −362	3 versus 2	0.15
4	MCP1−2581 × MCP1 −362	4 versus 3	0.16

Table 5. Transmission disequilibrium test

MCP-1 allele	Cases [*n* (%)] (frequency)	Controls [*n* (%)] (frequency)	OR 95% CI	P_{nom}	P_{corr}
−2581A	558 (85.1)	527 (80.3)	1		
−2581G	98 (14.9)	129 (19.6)	0.72 (0.53–0.98)	**0.04**	**0.04**
−362G	400 (60.6)	342 (51.9)	1		
−362C	260 (39.4)	318 (48.1)	0.70 (0.55–0.89)	**0.003**	**0.004**

TDT performed with 207 complete trios (affected child and both parents) and 125 pairs of an affected child with a single parent. P_{nom}, nominal *P*-value; P_{corr}, corrected *P*-value after 10 000 permutations.

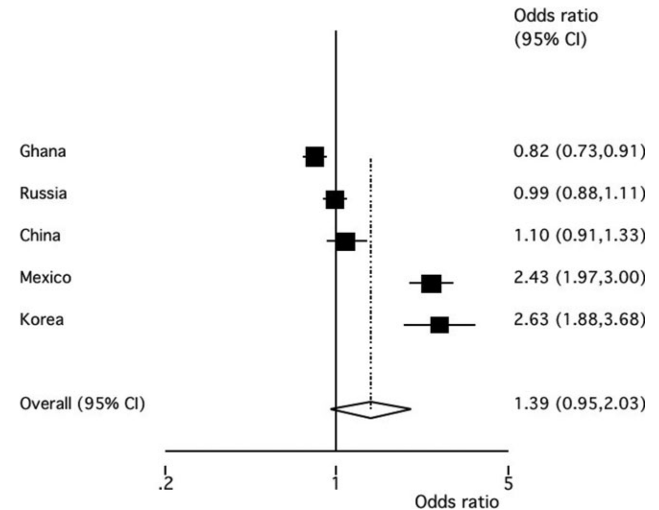

Figure 2. Meta-analysis of five case–control studies analysing the allelic association between the *MCP-1* variant 2581A>G and susceptibility to TB. Black squares represent the ORs of each study/ethnicity. The pooled OR ($P = 0.09$) is indicated by the diamond.

DISCUSSION

The functional significance of the MCP-1 chemokine in attracting monocytes to the site of infectious lesions and its presumed role in the pathogenesis of TB and the granulomatous response suggested that variation of the *MCP-1* gene might participate in conferring susceptibility to or protection from TB. This hypothesis prompted us to study several *MCP-1* polymorphisms in two case–control sample collections from Ghana and Russia, both of considerable size.

In contrast to the earlier report that suggested an association of *MCP-1* −2581G with susceptibility to TB in Mexicans and Koreans (4), we found an opposite association of *MCP-1* −2581G, namely with resistance to TB in the Ghanaian population and no effect at all on TB in the Russian sample. Taking into consideration our results and the fact that no association of the *MCP-1* −2581 polymorphism with TB has been observed in the Brazilian and Chinese populations (3,5), we believe that, at present, the overall data do not sufficiently support any major involvement of that variant in TB susceptibility or protection. This is supported by the meta-analysis that we performed applying the random effect model of the DerSimonian and Laird algorithm (Fig. 2). The comparison of the five pooled case–control studies did not show a significant contribution of *MCP-1* −2581 to TB susceptibility or to protection from disease ($P = 0.09$). However, as a considerable degree of heterogeneity was observed ($P < 0.001$), it may be assumed that variability exists in the LD patterns in the different populations that were reviewed in the meta-analysis.

We found another polymorphism, *MCP-1* −362, associated with protection from TB both in the Ghanaian case–control sample and in nuclear families. As the −2581 and −362 variants exhibited a moderate LD ($r^2 = 0.27$) in our study population, we tried to define which of the two variants caused the strongest effect and found in regression analyses that the −362CC genotype, and not the −2581 variant, exclusively explained the observed association with resistance to TB.

When reconstructing haplotypes comprising the variants −2581 and −362, none of the three haplotypes was associated with protection stronger than was the −362CC genotype alone. This was confirmed by interaction analyses indicating that, conditional on the −362 variant, no other variant improved the model predicting the phenotype. Our case–control results were corroborated by those of the modified TDT of nuclear families.

In order to compare our findings in the Ghanaian and Russian populations with previously published data, we analysed LDs between *MCP-1* polymorphisms. Pairwise *MCP-1* LDs of more than 4300 Ghanaian individuals participating in the present study (GHN), populations of Mexican ancestry from California (MXL), US Caucasian residents of northern and western European ancestry (CEU) and of the Nigerian Yoruba ethnicity (YRI) are depicted in Figure 1. The LD analysis provided an important insight. When comparing the linkage plot of our GHN study population to the plots of Caucasians and Mexicans (MXL) using preliminary data from the HapMap Phase 3 release, clear differences of LD patterns became evident. Comparisons of LDs in these ethnicities indicate that, in Ghanaians, the alleles at positions −2581 and

−362 are in moderate LD only with each other ($r^2 = 0.27$) as they are in Yoruba ($r^2 = 0.26$), whereas they are in perfect LD in Caucasians ($r^2 = 1.0$). MXL data on *MCP-1* −362 are not available so far. As in all populations, except our GHN and the YRI population, for which data are available in the HapMap database, −2581 is in strong LD with +5356 (data not shown), a corresponding LD between the three variants −2581, −362 and +5356 may plausibly be inferred for the MXL population. Notably, when plotting LD patterns with all available HapMap *MCP-1* variants, the resulting LD plots appear to differ among ethnicities even to a still higher degree (data not shown in Fig. 1).

The LD pattern that is shown in Figure 1A suggests that, in Ghanaians, the association of *MCP-1* −362C is independent of that of −2581G, which is also evident from our logistic regression analysis. In contrast, given the perfect LD in the CEU population as evident from the HapMap data, it is reasonable to expect that the −362 and −2581 alleles will be in perfect LD in the Russian population as well, and −362 would show no association with TB susceptibility/resistance, should we genotype this variant in the Russian sample.

Several factors might explain the disparity of associations that was observed in different studies in various populations. First, our evidence of an association of −362C and resistance to TB in the Ghanaian population ($P_{corr} = 0.00017$ in the case–control data set and $P_{corr} = 0.004$ in the nuclear families) does not completely exclude an association by chance, and, therefore, replication in a large statistically powerful sample collection is important. Alternatively, the association of *MCP-1* −362 might reflect the presence of a genuine association owing to LD with another, putatively causal polymorphism in the adjacent *MCP-1* gene region. Different LD patterns between such a causal, yet unidentified variant and the −362 SNP in the Ghanaian population, on the one hand, and the Russian and other populations (Chinese, Brazilian), on the other hand, might explain the lack of association that we and others observed in non-African populations. A meta-analysis of findings of the *MCP*-1 −362 variant is currently not feasible, as TB association data are not available for other than the Ghanaian study population. This is in agreement with the distinct LD patterns that exist in African and non-African populations. Further analyses of other polymorphisms in the *MCP-1* gene region are needed to address this question in more detail. Finally, we cannot exclude that environmental variance, including different *M. tuberculosis* complex genotypes occurring in different parts of the world, might influence the effects of genetic variation on susceptibility to TB. These factors might also have contributed to the discrepant results of different studies, including the original study of Mexicans and Koreans.

In conclusion, we have identified a genetic variant, *MCP-1* −362C, that contributes to protection from pulmonary TB. It remains, however, to be elucidated by re-sequencing and fine mapping whether this variant is truly causal or is only part of a haplotype that comprises the causative genetic variant. This again will require meticulous analyses of LD patterns in different ethnicities. Once unquestionably identified, functional studies will help to determine in more depth the role of *MCP-1* variants in their contribution to relative resistance to TB.

MATERIALS AND METHODS

Ghanaian and Russian study groups

Participants were consecutively enrolled in Ghana, West Africa, between September 2001 and July 2004 at Korle Bu Teaching Hospital in Accra, Komfo Anokye Teaching Hospital in Kumasi, plus 15 additional hospitals and polyclinics in Accra and Kumasi and at regional district hospitals. The case group consisted of 2010 HIV-negative individuals with newly diagnosed smear-/culture-positive pulmonary TB. Out of the total of 2346 control individuals, 1211 were unrelated personal contacts of cases and 1135 were community members from neighbouring houses or working contacts of cases. Cases and controls belonged to the ethnic groups of Akan (Ashanti, Fante, Akuapem), Ga-Adangbe, Ewe, all in the south of Ghana, and several other ethnic groups from northern Ghana. The proportions of ethnicities among patients and controls did not differ significantly. The male-to-female ratio in the total study group 1 was 0.58, and the mean age of participants was 33 years without gender differences.

Phenotyping of patients included the medical histories and documentation of major symptoms on structured questionnaires, physical examination, HIV-1/2 testing (Capillus, Trinity Biotech, Bray, Co Wicklow, Ireland), posterior–anterior chest X-rays, Ziehl–Neelsen staining of two independent sputum smears and culturing of *M. tuberculosis* on solid Loewenstein–Jensen medium with subsequent determination of mycobacterial species, fine-typing of genotypes by spoligotyping, IS*6110* fingerprinting and determination of drug resistance as described previously (9–12). Cases were HIV-negative and had characteristic radiological lesions of pulmonary TB. All patients were treated in the framework of the DOTS (Directly Observed Treatment Short-Course strategy) programme organized by the Ghanaian National Tuberculosis Programme.

A number of cases that were identified during the enrolment procedure were excluded for one or several of the following reasons: history of TB or of previous antimycobacterial treatment, HIV positivity, lost to follow-up or refusal after primary enrolment, age not consistent with the age of 6–60 as specified in the study protocol, predisposition to immunosuppression (alcoholism, drug use, diabetes, overt generalized disease) and inappropriateness for matching with controls for sex and age.

Characterization of controls included a medical history and clinical examination, posterior–anterior chest X-ray and a tuberculin skin test (Tuberculin Test PPD Mérieux, bioMérieux, Nürtingen, Germany). A total of 2217 individuals were PPD-positive and 129 individuals were PPD-negative. The controls had no radiological signs of actual or past pulmonary TB and no history of specific antimycobacterial treatment. In order to verify results in transmission disequilibrium tests, genotypes of 332 affected individuals and their parents (trios) or children with single-parents were studied. The family sample was only in part independent from the case–control sample. Further details of the recruitment procedure and the composition of the study group including the distribution of ethnicities have been described previously (12–15).

The study protocol was approved by the Committee on Human Research, Publications and Ethics, School of

Table 6. Variants selected for genotyping

MCP-1 variant	rs number	Selection criterion	Primer oligonucleotides	Sensor/anchor oligonucleotides
−11822G/A	rs9889296	Imputation	F-AGACTAGGCACTTAATTACTGTT R-CTTTGCATTCCTTTATGACAGC	S-CTGGCATTTACTTATTCATTCATTTGCCTATTTAT A-CTTCTTTGAATCTATCTTAGCACCTAGGACAGGG
−2581A/G	rs1024611	HapMap	F-TTCTCTCACGCCAGCAC R-TGACTTGGCCTTTGCATATATC	S-AAAGTGACAGCTGTCTGCCTC A-CACTTCTGCTCTGTCAAGAAAAGATGCCCTCCCCC
−2138A/T	rs1024610[a]	Frequency	F-CAGCATCTTTCAGCTTGT R-GTGTAGGAATTTCTTCCTAGGC	S-GGGAAACCTCTCTCTAATCAGTTAGTGC A-TCCTTTACCATGAACTTGGTGGACCGCATTCAATT
−2134T/G	rs3917885[a]	Frequency	F-CAGCATCTTTCAGCTTGT R-GTGTAGGAATTTCTTCCTAGGC	S-GGGAAACCTCTCTCTAATCAGTTAGTGC A-TCCTTTACCATGAACTTGGTGGACCGCATTCAATT
−1549A/T	rs3091332	Frequency	F-GCACATCTACTATTCTGTCTGAGTTA R-AGAAAACACTTTTCACTACACTTG	S-AAAGTATGTCAGCACCATACCTGACTCCCTGAATG A-ACTCAACAATGCCATTACTGACCAC
−362G/C	rs2857656	Frequency	F-GAGCCTGACATGCTTTCATCTA R-TTTCCATTCACTGCTGAGAC	S-TTCGCTTCACAGAAAGCAGAATCCTTA A-AAATAACCCTCTTAGTTCACATCTGTGGTCAGTCT
+900C/T	rs4586	HapMap	F-TAAGATCAGAATAATCCAGTTCATCC R-GCTGGTGATTCTTCTATAGCTC	S-TCACCTGCTGCTATAACTTCACCAA A-AGGAAGATCTCAGTGCAGAGGCTCGC
+3318C/T	rs3917891	HapMap	F-CTGGCAAGAAGCACACT R-CCTCTGCAACTCCAGTTAG	S-ACTCGCTTTGTCAGTCAAGACAGGTCAGATATTCT A-AGCCTACATCGATCATACAGGTATGATAAT
+5356C/T	rs991804	Imputation	F-GCTCCTCTTCCCATTGC R-GTCAGAACAAAGGACACTATGAAA	S-CAGCCAGTCCTGGTAACCTCTG A-TCCTCAGTTCTCTCATATTCAGGTCATTGGAGCCA

Primer pairs and sensor/anchor oligonucleotides for LightTyper-based *MCP-1* genotyping.
F, forward primer; R, reverse primer; S, sensor; A, anchor; Imputation, SNPs selected owing to an increased association with the phenotype after imputation of genotypes of so far untyped SNPs; HapMap, tagging SNPs from the HapMap database of the Yoruba ethnicity; Frequency, SNPs chosen according to high differences in frequencies in Caucasian and Asian ethnicities.
[a]Detected by the same PCR and genotyping assay.

Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, and the Ethics Committee of the Ghana Health Service, Accra, Ghana. Venous blood samples were taken only after a detailed explanation of the aims of the study, and consent was obtained by signature, or thumbprint in case of illiteracy.

One of the SNPs associated with protection in the Ghanaian sample was typed in an independent sample of 1440 TB patients and 1529 controls from Russia. Russian TB patients and controls were collected at St Petersburg. Patients [mean age 43.8 (17–86), 26.2% female] had been diagnosed by the local health care service using information provided on TB contact, clinical symptoms, chest X-rays and sputum smears. Diagnoses were confirmed by sputum culture of *M. tuberculosis* on solid and/or liquid culture medium. Clinical information, as well as age, ethnicity, socio-economic status and the concurrent medical history were recorded on structured questionnaires. Population controls were adult blood bank donors with no history of TB [mean age 30 (16–66), 25.0% female]. Ethical clearance was obtained from local ethics committees in St Petersburg, Russia, and Cambridge, UK, and written informed consent was provided by all participating subjects. Patients with extra-pulmonary TB and HIV-positive subjects were excluded. Further details on the Russian case–control group are given in Nejentsev *et al*. (16).

Variants selected for genotyping; genetic analyses

The variants, rs-numbers and PCR amplification primers as well as sensor/anchor nucleotides for genotyping are summarized in Table 6. First, seven *MCP-1* variants were selected for genotyping, among them five promoter, one exonic and one 3′ UTR variants. Three of the variants (rs1024611, rs4586, rs3917891) were tagging SNPs extracted from the HapMap database for the West African ethnicity of Yoruba (YRI), and four promoter variants (rs1024610, rs3917885, rs3091332, rs2857656) were chosen because of their high frequencies in Africans (http://innateimmunity.net//PGAs/InnateImmunity/CCL2/). As an additional exploratory analysis, genotypes were imputed for untyped HapMap (release 2) SNPs (YRI) of the *MCP-1* region using the software PLINK 1.0.3 (http://pngu.mgh.harvard.edu/~purcell/plink/download.shtml/). After imputation, two additional variants (rs9889296, rs991804) were selected because of an increased association with the phenotype studied.

DNA was extracted from whole blood according to standard methods. Genotypes of *MCP-1* variants were determined by dynamic allele-specific hybridization with fluorescence resonance energy transfer (LightTyper®; Roche Diagnostics, Mannheim, Germany). The sequences of sensor and anchor nucleotides of LightTyper-based genotyping are given in Table 6.

Databases and statistical analyses

Demographic data, self-reported signs and symptoms and primary laboratory results were double-entered into a 4th Dimension database (San José, CA, USA). Microbiological data were provided as datasheets. All data were locked before using them in a pseudonymized form for analyses.

Power calculation was performed with the public CATS software (http://www.sph.umich.edu/csg/abecasis/CaTS/). The STATA 9 software (Stata Corporation, College Station, TX, USA) was used to calculate HWE and ORs of *MCP-1* allele and genotype frequencies. Corrections of *P*-values were performed according to the Bonferroni–Holm procedure (17).

Notably, *MCP-1* −1549 (rs3091332) and +3318 (rs3917891) were in almost perfect linkage ($r^2 = 0.98$; Fig. 1).

Logistic regression analyses (STATA 9) were applied to adjust for gender, age and ethnicity and to calculate SNP–SNP effects. Haplotype frequencies and OR with global and adjusted *P*-values (10 000 permutations) were estimated and compared with the public UNPHASED software (version 3.0.12; http://www.mrc-bsu.cam.ac.uk/personal/frank/software/unphased/). LDs were calculated and graphically displayed with the Haploview 4.1 software (www.broad.mit.edu/mpg/haploview/). A logistic regression model was applied in an SNP–SNP effect and interaction analysis to test whether the effect of *MCP-1* −362 was independent of that of *MCP-1* −2581. Different models of single variants and the combination of two variants were compared with an LR test. Modified TDT with the implementation of maximum likelihood analyses were calculated to avoid biases caused by ethnic stratification (UNPHASED software). Genotypes of 207 full trios (affected child plus both unaffected parents) and 125 parent–child pairs (affected child plus a single unaffected parent, mostly the mother) were included in the TDT.

A meta-analysis of results available for the *MCP-1* −2581 genotype, including the present study of Ghanaians and Russians as well as the case–control studies of Mexican and Korean individuals (4) and of Hong Kong Chinese TB patients and controls (5), was conducted (STATA 9). The test of heterogeneity between the studies was assessed by a χ^2 statistic. The DerSimonian and Laird random effect model was applied estimating the pooled OR and CI.

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Conflict of Interest statement. None declared.

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