RESEARCH ARTICLE

Matrix metalloproteinase-1 promoter -1607 bp 1G/2G polymorphism associated with increased risk of spinal tuberculosis in Southern Chinese Han population

Ying Zhou¹ | Qile Gao² | Dan He³ | Ang Deng² | Rongfu Huang⁴ | Yanbing Li⁵ | Chunyan Tan¹ | Chaofeng Guo² | Qiang Guo² | Longjie Wang² | Guanteng Yang² | Hongqi Zhang²

¹Department of Clinical Laboratory, The People's Hospital of Guangxi Autonomous Region, Nanning, China

²Department of Spine Surgery, Xiangya Spinal Surgery Center, Xiangya Hospital, Central South University, Changsha, China

³Department of Neurology, The First Hospital of Changsha, Changsha, China

⁴Department of Clinical Laboratory, The Second Affiliated Hospital, Fujian Medical University, Quanzhou, China

⁵Department of Clinical Laboratory, Xiangya Hospital, Central South University, Changsha, China

Correspondence

Chaofeng Guo, Department of Spine Surgery, Xiangya Spinal Surgery Center, Xiangya Hospital, Central South University, Changsha, China.

Email: chaofengguoxy@hotmail.com

Funding information

National Natural Science Foundation of China, Grant/Award Number: 81401818 and 81301576; medical and health planning project of Guangxi Zhuang Autonomous Region Health and Family Planning Commission, China, Grant/Award Number: Z2016611

Background: Spinal tuberculosis is the most common form of musculoskeletal tuberculosis. The expression of matrix metalloproteinase-1 (MMP-1) is increased in cells with *Mycobacterium tuberculosis* infection. MMP-1 plays a curial role in extracellular matrix degradation during the progression of tuberculosis. Although the 1G/2G polymorphism in *MMP-1*-1607 influences its transcription, its role in spinal tuberculosis remains unknown.

Methods: Healthy controls and patients with spinal tuberculosis of Han ethnicity were recruited between January 2010 and May 2016. The *MMP-1*-1607 1G/2G polymorphism was genotyped using the Sequenom mass Array polymorphism analysis system.

Results: The genotypes of 1G/1G, 1G/2G, and 2G/2G were found in 13.7%, 53.6%, and 32.8% of patients, and 12.2%, 37.4%, and 50.4% of controls, respectively. The 1G/2G genotype were more common in cases than in controls ($P=2.05E-04$). The 1G allele showed an association with an increased risk for spinal tuberculosis when compared to 2G allele ($P=.004$). 1G genotypes, having at least one 1G allele, were associated with the risk of developing spinal tuberculosis (1G/1G+1G/2G vs 2G/2G: OR=2.084, 95%CI=1.401-3.100, $P=2.65E-04$).

Conclusion: 1G genotypes of the *MMP-1*-1607 may be associated with susceptibility to spinal tuberculosis in Southern Chinese Han population.

KEYWORDS

matrix metalloproteinase-1, polymorphism, Southern Chinese Han population, spinal tuberculosis

1 | INTRODUCTION

According to the WHO Global Tuberculosis Report 2015,¹ there were approximately 9.6 million patients with tuberculosis (TB) worldwide in 2014. China accounted for 10% of the global TB burden, with the third highest incidence and prevalence of TB worldwide.

The spine is a common extra-pulmonary site of TB infection. Spinal TB occurs in 1%-5% of all TB cases, and accounts for 50% of musculoskeletal TB cases.^{2,3} Spinal TB is usually secondary to pulmonary or abdominal lesions. *Mycobacterium tuberculosis* (MTB) spreads from the initial loci into vertebral bodies or intervertebral discs via the hematogenous or lymphatic routes.⁴

Excessive matrix metalloproteinase-1 (MMP-1) activity is likely to increase the risk of MTB dissemination. In transgenic mice expressing human MMP-1, MTB infection induced increased MMP-1 gene expression and MMP-1 secretion, leading to remarkable granuloma destruction and alveolar collagen breakdown.⁵ Microarray profiling has confirmed that MMP-1 expression in granulomas was up-regulated 606-fold compared with uninfected lung tissue.⁶ Granulomas are considered beneficial to the host by containing bacilli and restricting MTB expansion.⁷ However, the collagenase activity of MMP-1 may destabilize the formation of new granulomas and damage adjacent tissues in TB, thus contributing to the dissemination of infection and disease progression.^{8,9}

Matrix metalloproteinase-1 is potentially involved in spinal disc destruction. Xiong et al.¹⁰ showed increased expression of MMPs (MMP-8, -9, -13, and -14) in spinal TB intervertebral discs (ID) tissue compared to normal ID tissue, and suggesting that the co-expression of MMPs may result from the MMP cascade. Moreover, Song et al.¹¹ found an association between the MMP-1-1607 promoter polymorphism and degenerative disc disease. This may be due to linkage disequilibrium, with MMP-1 located on the MMP genes cluster.¹²

A single nucleotide polymorphism (SNP) in the MMP-1 promoter region (-1607 bp), where an insertion of a guanine (G) creates a core-binding site (5'-GGA-3') for the Ets transcription factors, may affect the transcription level of MMP-1 gene.^{13,14}

Whether MMP-1 gene polymorphisms alter spinal TB risk remains unknown. This study therefore aims to determine the association between the MMP-1-1607 1G/2G polymorphism and susceptibility to spinal TB in Southern Chinese Han population.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

This study was approved by the Ethical Committee of Central South University, China. Signed informed consent was obtained from all participants in accordance with the Helsinki Declaration.

2.2 | Patients and controls

All subjects were Han ethnicity and residing in Southern China. The study was performed in Xiangya Hospital, The People's Hospital of Guangxi Autonomous Region, The Second Affiliated Hospital of Fujian Medical University, and The First Hospital of Changsha from January 2010 to May 2016. Patients' relevant information was assessed, including individual medical history, previous medical records or examination results during hospitalization. The patients or controls who met the exclusion criteria would not be included in the study.

In this study, we included patients with spinal TB and healthy controls. The diagnosis and differential diagnosis of TB was mainly depended on microbiological and/or pathohistological examination with sample from the focus and the anti-TB treatment was effective. All spinal TB patients underwent surgical treatment and received postoperative anti-TB chemotherapy. They were followed up for more than 3 months for outcome assessment.

2.3 | Inclusion criteria

Patients with spinal TB presenting with all of the following were considered for admission:

1. Clinical manifestations: chronic back or neck pain, fever, weakness, abscesses or sinus tract formation, spinal localized tenderness; complications including neurologic deficit, paraplegia, kyphosis, sensory disturbance, bowel, and bladder dysfunction.
2. Laboratory examination: positive T-SPOT TB test results, increased C-reactive protein (CRP) level and erythrocyte sedimentation rate.
3. Microbiological or histological examination: bone biopsy (with or without biopsies of the paraspinal abscess) results showing positive Ziehl-Neelsen (ZN)-stained smears and positive Lowenstein-Jensen (L-J) culture. Pathohistological findings of bone marrow biopsy suggestive of TB include either caseating or noncaseating granuloma formation.
4. Radiographic examination (MRI or CT scans): bone destruction, disc space narrowing, presence or absence of cold abscesses in adjacent structures, compression of the spinal cord or spinal nerve roots.

Age-, ethnicity- and sex-matched healthy controls were recruited from the same geographical area. Healthy control subjects had no history of TB, no evidence of past exposure to TB and no evidence of pulmonary or extra-pulmonary TB by imaging. In addition, healthy controls had a negative result of PPD skin test, T-SPOT TB test, and TB-IgG/IgM.

2.4 | Exclusion criteria

Exclusion criteria for patients and controls were as follows: individuals who had not received Bacillus Calmette-Guérin vaccine; individuals with disease impairing the immune system (infection, trauma, and tumor), genetic disease or autoimmune disease; individuals using hormones or immune inhibitors; and individuals who had a positive result of PPD skin test/or T-SPOT TB test/or TB-IgG/IgM.

2.5 | Specimen collection and genomic DNA extraction

Genomic DNA for sequencing was extracted from peripheral blood, using the Takara kit (Takara, Dalian, China) according to the manufacturer's instructions.

2.6 | Primers

Primers for MMP-1 were designed and synthesized using Mass Assay Designer 3.1 software (Sequenom, San Diego, CA, USA). The sequences of the PCR primers were 5'-ACGTTGGATGGTTATGCCACTTAGATGAGG-3' (forward primer) and 5'-ACGTTGGATGCTTCAGTATATCTTGATTG-3' (reverse primer); the PCR product was

104 bp in length. The single base extension primer used was 5'-TTGG ATTGATTGAGATAAGTCATATC-3'.

2.7 | Genotyping

PCR reactions were set up in a 5 μ L reaction volume with 10 ng genomic DNA, 0.5 μ L 10 $\times$ PCR Buffer (Sequenom), 10 nmol $MgCl_2$, 0.5 pmol Primer Mix, 1 U HotStarTaq (Takara), 2.5 nmol deoxy-ribonucleoside triphosphate (dNTP) Mix (Takara), and 1.8 μ L double-distilled water. The thermal cycling program was: 94°C for 15 minutes; 45 cycles of 94°C for 20 seconds, 56°C for 30 seconds and 72°C for 1 minute; and a final extension at 72°C for 3 minutes.

PCR products were purified with Shrimp Alkaline Phosphatase and extension reactions were performed according to the manufacturer's (Sequenom) protocol.

Purified extension reaction products were spotted onto SpectroCHIPs (Sequenom) after being desalted with SpectroClean resin (Sequenom).

Genotyping was performed by using the Mass Spectrometry platform (Sequenom).

2.8 | Statistical and genetic analyses

Data were analyzed using the SPSS statistical package (SPSS 22.0 for Windows; SPSS Inc., Chicago, IL, USA). Demographic characteristics of the studied population were compared using *t* tests and χ^2 tests. The results of *t* tests were reported as means $\pm$ standard deviation (SD). The χ^2 test was used to determine the Hardy-Weinberg equilibrium (HWE) of the genotype distribution, and the frequencies of genotypes and alleles between groups were compared using the χ^2 test. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. A *P*-value <.05 was considered statistically significant.

3 | RESULTS

3.1 | Cohort demographics

A total of 183 patients with spinal TB (95 male and 88 female; mean age 39.1 $\pm$ 18.9 years) and 246 healthy controls (107 male and 139 female; mean age 40.4 $\pm$ 10.4 years) were recruited for this study. There were no significant differences in gender ($\chi^2=2.984$, *P*=.084) or age (*t*=-0.573, *P*=.568) distributions between patients and controls.

3.2 | Hardy-Weinberg equilibrium test

The distribution frequencies of the 1G/1G, 1G/2G, and 2G/2G genotypes are shown in Table 1. The genotype distributions of MMP-1-1607 in cases and controls were confirmed with Hardy-Weinberg equilibrium (*P*>.05).

3.3 | Association of the MMP-1-1607 1G/2G polymorphism with spinal TB

Genotype distributions and allele frequencies for the MMP-1 promoter -1607 1G/2G polymorphism are summarized for cases and controls in Table 1.

The frequency distributions of the 1G/1G, 1G/2G, and 2G/2G genotypes were 13.7%, 53.6%, and 32.8% in cases, and 12.2%, 37.4%, and 50.4% in controls, respectively. Significant differences were observed in the genotype distributions between the two groups (*P*=9.33E-04). The 1G/2G genotype was more common in cases than in controls. Paired comparisons of genotypes showed that the risk of spinal TB was higher in individuals with the 1G/2G genotype compared with those carrying the 2G/2G genotype (OR=2.201, 95% CI=1.447-3.349, *P*=2.05E-04).

TABLE 1 Genotype and allele frequency distributions of MMP-1-1607 polymorphism in patients with spinal TB and controls

MMP-1-1607		Patients with spinal TB n=183		Controls n=246		P-value	OR	95%CI
Genotypes	1G/1G	25	13.7%	30	12.2%	.081 ^a	1.722	0.932-3.182
	1G/2G	98	53.6%	92	37.4%	2.05E-04 ^b	2.201	1.447-3.349
	2G/2G	60	32.8%	124	50.4%		1	Ref
Alleles	1G	148	40.4%	152	30.9%	.004 ^c	1.519	1.144-2.016
	2G	218	59.6%	340	69.1%		1	Ref
Dominant model (1G genotypes)	1G/1G+1G/2G	123	67.2%	122	49.6%	2.65E-04 ^d	2.084	1.401-3.100
	2G/2G	60	32.8%	124	50.4%		1	Ref
Recessive model	1G/1G	25	13.7%	30	12.2%	.653 ^e	1.139	0.645-2.013
	1G/2G+2G/2G	158	86.3%	216	87.8%		1	Ref

CI, confidence interval; OR, odds ratio; Ref, reference.

^a*P*>.05 case vs control, comparing 1G/1G genotype with 2G/2G genotype, by χ^2 test.

^b*P*<.05 case vs control, comparing 1G/2G genotype with 2G/2G genotype, by χ^2 test.

^c*P*>.05 case vs control, comparing 2G allele with 1G allele, by χ^2 test.

^d*P*<.05 case vs control, comparing 1G genotypes (1G/1G genotype and 1G/2G genotype) with 2G/2G genotype, by χ^2 test.

^e*P*>.05 case vs control, comparing 1G/1G genotype with 1G/2G genotype and 2G/2G genotype, by χ^2 test.

The frequencies of 1G and 2G alleles were 40.4%, 59.6%, in cases, and 30.9%, 69.1%, in controls, respectively. Analysis of allelic distributions revealed that the 1G allele was related to increased risk of spinal TB when compared to 2G allele (OR=1.519, 95%CI=1.144-2.016, $P=.004$).

Association analyses were performed to assume both dominant and recessive models of inheritance, as shown in Table 1. 1G genotypes, having at least one 1G allele, were associated with the risk of developing spinal TB (1G/1G+1G/2G vs 2G/2G: OR=2.084, 95%CI=1.401-3.100, $P=2.65E-04$).

4 | DISCUSSION

MMP-1 gene expression and protein product secretion are MTB-specific in human monocytes and macrophages. Chang et al.¹⁵ found that MTB and its components up-regulated the level of *MMP-1* mRNA in human peripheral blood monocytes in vitro; while Elkington et al.¹⁶ showed that MTB was a more potent stimulus to human macrophage *MMP-1* expression than the vaccine *Bacillus Calmette-Guérin* (BCG).

Collagen is an essential component of lung ECM and of MTB-induced granulomas. Both granuloma breakdown and alveolar collagen destruction result in TB dissemination,^{17,18} allowing MTB to spread hematogenously from primary pulmonary loci to vertebral bodies or discs.⁴ Collagen is also the major component of the inner nucleus pulposus and outer annulus fibrosus in the intervertebral disc.¹⁹ Overexpression of *MMP-1* is associated with increased ECM destruction, leading to the breakdown of disc components. All these factors favor the progression of spinal TB.

Regulation of *MMP-1* activity is a complex process. A polymorphism located in the promoter region (-1607 bp) of *MMP-1*, which potentially alters *MMP-1* gene expression has drawn much attention in recent years.^{13,14} Ju et al.²⁰ showed that the frequency of *MMP-1*-1607 1G/1G genotype was relatively low among Asians. We made a similar observation in a Han population of Southern China, where the frequencies of the 1G/1G genotype were 13.7% in cases and 12.2% in controls, respectively. Interestingly, we found that *MMP-1*-1607 1G/2G genotype and 1G allele were more frequent in spinal TB patients than in control subjects ($P<.05$). And our data also indicated that carriers of the 1G genotypes had a 2.084-times increased risk of developing spinal TB (95%CI=1.401-3.100, $P=2.65E-04$).

Researches on the role of the 1G allele in other diseases have yielded similar findings. Ninomiya et al.²¹ reported that the 1G/1G or 1G/2G genotype was significantly more common in sarcoidosis patients with ocular or multi-organ involvement, and that the 1G/1G genotype was more common in pulmonary TB patients with cavity formation.

The 1G allele may therefore increase the risk of developing TB and contribute to disease progression. Kuo et al.²² found significantly increased expression of *MMP-1* in bronchial epithelium, infiltrating pro-inflammatory cells, and submucosal granulomas by immunohistochemistry analysis. Moreover, pulmonary TB patients carrying the 1G genotypes were more vulnerable to endobronchial TB (OR=2.813,

95%CI=1.114-7.099, $P=.026$). Wang et al.²³ also found that the 1G allele was correlated with the severity of fibrosis in a Taiwanese pulmonary TB cohort. Pulmonary TB patients carrying the 1G genotypes have an increased risk of moderate (OR=5.04, 95%CI=1.25-20.30) and advanced fibrosis (OR=9.87, 95%CI=2.39-40.88).

Tumor cells homozygous for the 1G allele have high levels of *MMP-1* expression,²⁴ which in VMM5 melanoma cells, is mediated by both the p38 and ERK1/2 signaling. In the absence of the 2G allele, *MMP-1* expression is compensated by an alternative pathway with cis-acting sequences to achieve high levels of *MMP-1* expression, which promotes matrix degradation, and subsequent MTB dissemination.

Similarly, increased expression of *MMP-1* has been noted in other cells homozygous for the 1G allele. Kuo et al.²² and Wang et al.²³ found that interleukin-1 β enhances *MMP-1* secretion in peripheral blood monocytes in TB patients with 1G genotypes. Monocyte/macrophages are the major effector cells preventing MTB infection. Therefore, the 1G genotype is plausibly responsible for up-regulating *MMP-1* expression, which leads to the expanding infection and development of TB.

However, the role of 1G genotypes as TB risk factors is still under debate. For example, Ganachari et al.⁸ identified the *MMP-1* 2G allele and 2G/2G genotype as risk factors for pulmonary TB. These contradictory findings could be explained by one of three possible scenarios: First, the site of disease varied between our study (spinal TB) and that of Ganachari et al. (mainly pulmonary TB). Different pathological mechanisms and host immune responses between pulmonary and spinal TB may be linked to different susceptibility factors. Second, linkage disequilibrium played a role in the study of Ganachari et al.; they found that the *MCP-1*-2518 GG genotype and *MMP-1*-1607 2G/2G genotype had joint effects, with the two loci resulting in increased *MMP-1* expression. Third, race may explain the differential effects seen between studies, as subjects in the study of Ganachari et al.⁸ were Mexicans and Peruvians. Diversity of environment and genetic background may influence host susceptibility to MTB.

The pathogenesis of spinal TB is complex, and this study shows that *MMP-1*-1607 1G genotypes likely increase the risk of spinal TB. Related studies are underway. Larger cohorts together with animal experiments will aid in clarifying the mechanism whereby the *MMP-1* genotype influences susceptibility to spinal TB. The effects of different genotypes on *MMP-1* transcriptional activity, protein expression and related molecular mechanisms should be further investigated.

5 | CONCLUSIONS

In summary, our findings suggest that *MMP-1*-1607 1G genotypes predispose the Han population of Southern China to spinal TB.

ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China (Grant No.81401818, 81301576) and medical and health

planning project of Guangxi Zhuang Autonomous Region Health and Family Planning Commission, China (No. Z2016611).

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How to cite this article: Zhou Y, Gao Q, He D, et al. Matrix metalloproteinase-1 promoter -1607 bp 1G/2G polymorphism associated with increased risk of spinal tuberculosis in Southern Chinese Han population. *J Clin Lab Anal*. 2017;31:e22136. <https://doi.org/10.1002/jcla.22136>