Functional polymorphisms in the promoter region of MMP-2 and MMP-9 and susceptibility to obstructive sleep apnea

Chao Cao1,2, Bin Wu3, Yanping Wu2, Yiming Yu1, Hongying Ma1, Shifang Sun1, Qiaoli Zhang1, Qunli Ding1, Li Chen4 & Zaichun Deng1

1Department of Respiratory Medicine, Affiliated Hospital of School of Medicine, Ningbo University, Ningbo 315020, China, 2Department of Respiratory and Critical Care Medicine, Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310009, China, 3Department of Respiratory and Critical Care Medicine, Affiliated Hospital, Institute of Respiratory Diseases, Guangdong Medicine College, Zhanjiang 524000, China, 4Department of Medical Oncology, First Affiliated Hospital of Nanchang University, Nanchang 330006, China.

Genetic susceptibility to obstructive sleep apnea (OSA) has been a research focus in the scientific community in the past few years. In this study, we recruited 375 subjects to investigate whether functional polymorphisms in the promoter region of matrix metalloproteinase (MMP)-2 (-1306C/T) and MMP-9 (-1562C/T) increased susceptibility to OSA. Our study showed no significant association between MMP-2 -1306C/T polymorphism and risk of OSA (T vs. C: OR = 1.01, 95% CI = 0.67–1.52; P = 0.97). Compared with the MMP-9 -1562C allele, the -1562T allele was associated with increased risk of OSA (T vs. C: OR = 1.56, 95% CI = 1.02–2.39; P = 0.04). However, neither MMP-2 -1306C/T nor MMP-9 -1562C/T polymorphism was found to be associated with severity of the disease. Our study suggested that the MMP-2 -1306C/T polymorphism was not associated with OSA susceptibility, whereas the MMP-9 -1562T allele was associated with increased risk of OSA.

Obstructive sleep apnea (OSA) is a prevalent sleep disorder characterized by repeated events of partial or complete upper airway obstruction during sleep, which results in intermittent hypoxemia, sleep fragmentation, and daytime sleepiness1. OSA has also been associated with cardiovascular diseases, such as hypertension, coronary heart disease, heart failure, stroke, cardiac arrhythmias, and sudden cardiac death2. OSA is a common disease with high morbidity in both adults and children, and the underlying mechanism of the disease remains unknown. In the last few years, a growing body of evidence has suggested that matrix metalloproteinases (MMPs) are involved in the pathogenesis of OSA3–12. A common polymorphism of MMP-2 gene is (-1306C/T) [rs243865], which is found to be associated with a strikingly lower activity with the T allele13. At the same time, there is evidence that -1562C/T polymorphism in the promoter region of MMP-9 [rs3918242] is associated with its mRNA transcription and protein production14. However, the relationship between MMP-2 and MMP-9 polymorphisms and risk of OSA has not been investigated worldwide. Based on the pathologic significance of MMPs in OSA and the potential biological effects of MMP-2 (-1306C/T) and MMP-9 (-1562C/T) polymorphisms on their protein expression, we hypothesized that functional polymorphisms in the promoter region of MMP-2 and MMP-9 would be associated with differential risk of OSA. Therefore, we conducted a case-control study to investigate whether polymorphisms of MMP-2 (-1306C/T) and MMP-9 (-1562C/T) increased susceptibility to OSA in a Chinese population.

Results

Genotype frequencies and risk of OSA. The MMP-2 -1306C/T and MMP-9 -1562C/T genotypes observed in controls were consistent with Hardy–Weinberg equilibrium (HWE) (P > 0.05, chi-squared goodness of fit). Allele frequencies of MMP-2 -1306C and MMP-9 -1562C polymorphisms in the controls were 0.85 and 0.89, respectively, which were in close agreement with frequencies previously observed in healthy Chinese individuals (0.83 and 0.90, respectively)15. The genotype distributions and allele frequencies of MMP-2 -1306C/T and MMP
MMP-2 -1306C/T polymorphism and risk of OSA (T vs. C: OR = 1.2, 95% CI = 0.7–2.1; P = 0.4). Interestingly, subjects with the MMP-9 -1562T allele were associated with an increased risk of OSA when compared with -1562C carriers (OR = 1.56, 95% CI = 1.02–2.39). Significant associations between the MMP-9 -1562C/T polymorphism and risk of OSA were also observed under heterozygote comparison (CT vs. CC: OR = 1.86, 95% CI = 1.15–3.01) and dominant genetic model (CT/TT vs. CC: OR = 1.78, 95% CI = 1.11–2.85). One possible explanation for these results is that DNA sequence variations in the MMP-9 gene may alter its protein production and/or activity. The investigators observed that T allele-associated promoter activity was higher than the C allele-associated promoter activity by transient transfection and DNA-protein interaction assays14. Moreover, published evidence has demonstrated that a genetic variant in the region of the MMP-9 -1562C/T polymorphism is associated with serum levels of MMP-924–26. The MMP-9 -1562C/T polymorphism associated with a predisposition to increased serum MMP-9 levels. Thus, it is biologically plausible that the functional polymorphism in this gene increases circulating MMP-9 expression, thereby causing individual differences in the development of OSA.

Some limitations of this study should be acknowledged. Firstly, in this study, we only investigated two functional polymorphisms in the promoter region of MMP-2 and MMP-9 with the risk of OSA. The association between other polymorphisms in MMP-2 and MMP-9 and their relationships to OSA susceptibility requires further study.

### Table 1 | MMP-2 -1306C/T and MMP-9 -1562C/T genotype and allele frequency in OSA patients and controls

| Polymorphisms | Patients (%) | Controls (%) | OR (95% CI) | P value |
|---------------|--------------|--------------|-------------|---------|
| MMP-2 -1306C/T |              |              |             |         |
| C/C           | 107 (71.3)   | 164 (72.9)   | 1.00 (Reference) | 0.61    |
| C/T           | 41 (27.4)    | 55 (24.4)    | 0.89 [0.56, 1.41] | 0.42    |
| T/T           | 2 (1.3)      | 6 (2.7)      | 0.51 [0.10, 2.58] | 0.74    |
| C/T and T/T   | 43 (28.7)    | 61 (27.1)    | 1.08 [0.68, 1.71] | 0.97    |
| Allele        |              |              |             |         |
| C             | 255 (85)     | 383 (85.1)   | 1.00 (Reference) | 0.04    |
| T             | 45 (15)      | 67 (14.9)    | 1.01 [0.67, 1.52] | 0.02    |
| MMP9 -1562C/T |              |              |             |         |
| C/C           | 103 (68.7)   | 179 (79.6)   | 1.00 (Reference) |         |
| C/T           | 46 (30.6)    | 43 (19.1)    | 1.86 [1.15, 3.01] | 0.01    |
| T/T           | 1 (0.7)      | 3 (1.3)      | 0.58 [0.06, 5.64] | 0.64    |
| C/T and T/T   | 47 (31.3)    | 46 (20.4)    | 1.78 [1.11, 2.85] | 0.02    |
| Allele        |              |              |             |         |
| C             | 252 (84)     | 401 (89.1)   | 1.00 (Reference) | 0.04    |
| T             | 48 (16)      | 49 (10.9)    | 1.56 [1.02, 2.39] |         |

MMP: matrix metalloproteinase; OSA: obstructive sleep apnea; OR = odds ratio; CI = confidence interval; NA: not applicable.
MMP-2 (-1306C/T) and MMP-9 (-1562C/T) polymorphisms were determined by samples with absorbance ratios from 1.8 to 2.0 at the length of A260/A280. The isolation kit (Tiangen, Beijing, China) according to the manufacturer’s protocol. For each case and control subjects were collected in EDTA-containing tubes. Genomic DNA isolation and genotyping assays were performed in accordance with American Academy of Sleep Medicine Guidelines1. Each participant was assessed by a detailed clinical interview and physical examination. Severity of disease was assessed based on the apnea hypopnea index (AHI), defined as the mean number of apneas and hypopneas per hour during sleep. OSA patients were categorized into 2 groups based on AHI: mild-to-moderate OSA (5 ≤ AHI < 30) and severe OSA (AHI ≥ 30). A total of 150 patients (F36: M114; age: 48.7 ± 11.5 years) with OSA and 225 healthy controls matched for age, sex, and ethnicity were included in this study. Base on AHI values, there were 82 patients with mild-to-moderate OSA (54.7%) and 68 with severe OSA (45.3%).

Polysonomography (PSG). All the subjects underwent an overnight laboratory-based PSG, which was analyzed according to recommendations published by the American Academy of Sleep Medicine1. Measurement channels included nasal pressure airflow, oxygen saturation (SpO2), heart rate, electroencephalogram, electrocardiogram, electrooculogram, electromyo-graph (chin and leg), snoring, and chest and abdominal movements. Participants went to bed before 23:00, and the recording was terminated after 6:00. The records were reviewed manually by trained sleep technologists for sleep stage, leg movements, arousals, apneas, and hypopneas. Records showing ≥ 4 hours of good quality respiratory signals were considered acceptable.

DNA isolation and genotyping assays. Five milliliters (5 ml) of venous blood from each case and control subjects were collected in EDTA-containing tubes. Genomic DNA was extracted from whole blood by using a commercially available DNA isolation kit (Tiangen, Beijing, China) according to the manufacturer’s protocol. Then, the purity of DNA was measured by means of absorption spectrometry, and the samples with absorbance ratios from 1.8 to 2.0 at the length of A260/A280. The MMP-2 (-1306C/T) and MMP-9 (-1562C/T) polymorphisms were determined by polymerase chain reaction – restricted fragment length polymorphism (PCR-RFLP) assays as previously described27,28. Briefly, PCR reactions were performed in a 20 μl reaction volume and cycle conditions consisted of an initial denaturation step at 94°C for 5 min, followed by 30 cycles of 30 s at 94°C, 30 s at 62°C, 30 s at 72°C, and then a final elongation step at 72°C for 5 min. The restriction enzymes for MMP-2 (-1306C/T) and MMP-9 (-1562C/T) genotypes were BstEII and NlaIII, respectively. PCR products were electrophoresed on a 3% agarose gel and visualized under UV light after staining with ethidium bromide.

Statistical analysis. The numbers observed for each genotype were compared with those expected for Hardy–Weinberg equilibrium using the χ2 test. The results were presented as the means ± standard deviation (SD) for all variables that were normally distributed and as median (interquartile range) when not normally distributed. If the data distribution was normal, comparison between different groups was done using Student’s t-test; otherwise, the nonparametric Mann-Whitney U-test was applied. The OSA risk associated with MMP-2 (-1306C/T) and MMP-9 (-1562C/T) genotypes was estimated by computing the odds ratios (ORs) and their 95% confidence intervals (CIs) by logistic regression analysis. Statistical analyses were conducted using SPSS (version 13.0, Chicago, III., United States) and GraphPad Prism 5.0 (GraphPad Software, San Diego, CA). A two-tailed P value < 0.05 was considered statistically significant.

Acknowledgments
This work was supported by grant of National Science Foundation of Ningbo (No. 2012A610257, Z. Deng).

Author contributions
C.C., Z.D. and L.C. designed the experiments. C.C., B.W., Y.W., Y.Y., H.M., S.S., Q.Z., Q.D., Z.D. and L.C. carried out the experiments and calculations. C.C., B.W., Y.W., Z.D. and L.C. wrote and edited the paper.
Additional information

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Cao, C. et al. Functional polymorphisms in the promoter region of MMP-2 and MMP-9 and susceptibility to obstructive sleep apnea. Sci. Rep. 5, 8966; DOI:10.1038/srep08966 (2015).

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder in order to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/