Associations of MMP-7 and OPN gene polymorphisms with risk of coal workers’ pneumoconiosis in a Chinese population: a case-control study

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Abstract

Background: The matrix metalloproteinase-7 (MMP-7) and osteopontin (OPN) are both multifunctional proteins with roles in inflammation, cell proliferation, tissue remodeling and so on, implicated in the pathogenesis of numerous conditions including pulmonary fibrosis. In this study, we investigated the associations between the potential functional polymorphisms in MMP-7 and OPN and the risk of coal workers’ pneumoconiosis (CWP) in a Chinese population.

Methods: Four polymorphisms (rs10502001 in MMP-7, rs1126772, rs11728697 and rs9138 in OPN) were genotyped and analyzed in a case-control study of 697 CWP and 694 control subjects.

Results: Our results revealed that three single nucleotide polymorphisms (SNPs, MMP-7 rs10502001, OPN rs1126772 and OPN rs11728697) were associated with increased risk of CWP under a recessive model (adjusted odds ratio [OR] = 1.80, 95% confidence interval [CI] = 1.01–3.20, p = 0.045 for MMP-7 rs10502001; adjusted OR = 2.09, 95% CI = 1.17–3.72, p = 0.013 for OPN rs1126772; adjusted OR = 2.48, 95% CI = 1.37–4.51, p = 0.003 for OPN rs11728697). Additionally, a combined effect was observed in a dose-dependent manner with increasing numbers of risk variant alleles (P_trend = 0.003). Furthermore, logistic regression analysis revealed no significant interaction between SNPs and smoking status on CWP risk.

Conclusions: Our results indicate that three functional SNPs (MMP-7 rs10502001, OPN rs11728697 and OPN rs1126772) are associated with an increased risk of CWP in a Chinese population.

Introduction

Coal workers’ pneumoconiosis (CWP) is an incurable occupational lung disease which is caused by long-term inhalation of airborne coal mining dust usually containing free crystalline silica (Chen et al., 2012; McCunney et al., 2009). In China, coal accounts for 60% of the primary energy source, and there are about 6 million underground coal workers who are all at risk of pneumoconiosis (Liu et al., 2007). Nowadays, CWP is still believed to be one of the most serious occupational diseases in China, and its pooled prevalence was 6.02% in Mo’s meta-analysis of the extracted data about the prevalence of CWP in 11 published reports from 2001 to 2011, much higher than that in the UK (0.8%, during 1998–2000) or USA (3.2% in the 2000s) (Mo et al., 2014).

Chronic exposure to coal particles results in macrophage-lymphocytic granulomatous lung inflammation, which is followed by abnormal and progressive accumulation of the extracellular matrix (ECM) (Rom, 1990). Cytokines play a role in a wide spectrum of biological processes, especially in inflammation and immune response, and are important fibrotic mediators of the toxic and pathogenic effects observed in humans exposed to mineral dusts (Schins & Borm, 1999). Development of fibrosis, characterized by an excessive deposition of ECM in the interstitium, where fibroblasts play a major role in the continuous and disbalanced tissue turnover and remodelling by producing new ECM components, is regulated by various proteolytic enzymes, including the matrix metalloproteinases (MMPs) (Nkyimben et al., 2013). Although respirable dust is the major contributory factor of CWP pathogenesis, not all individuals exposed to similar levels of coal dust develop the disease, suggesting that there is a genetic predisposition to the development of CWP (Yucesoy & Luster, 2007). Therefore, exploration of new genetic factors for CWP is crucial to the identification of high-risk individuals as well as prevention and treatment of CWP.

MMP-7, also called matrilysin 1, is a member of MMP gene family, which is a family of zinc-containing endopeptidases with roles in regulating abnormal epithelial response to injury, fibroblast proliferation, ECM accumulation and aberrant tissue remodeling (Pardo & Selman, 2006;
Pardo et al., 2008; Suga et al., 2000; Zuo et al., 2002). Moreover, MMP-7 has multiple local inflammatory regulatory roles (Li et al., 2002; McGuire et al., 2003). Lung gene expression microarray studies (Konishi et al., 2009; Selman et al., 2006; Zuo et al., 2002) have consistently identified MMP-7 to be one of the most upregulated genes in fibrotic lungs compared with normal lungs, and MMP-7 is elevated in the serum of idiopathic pulmonary fibrosis (IPF) patients (Richards et al., 2012; Rosas et al., 2008). Osteopontin (OPN), encoded by secreted phosphoprotein 1 (SPP1) gene, is a secreted phosphorylated multifunctional glycoprotein regulating inflammation and immune response, cell migration and proliferation, granuloma formation and tissue remodeling (Giachelli & Steitz, 2000; Mazzali et al., 2002; O’Regan & Berman, 2000; O’Regan et al., 2000; Pardo et al., 2005), with a unique effect on T cell function (Denhardt et al., 2001; O’Regan & Berman, 2000). OPN increases in bleomycin-induced lung injury in mice (Berman et al., 2004, Takahashi et al., 2001) and is elevated in the lungs and serum of patients with IPF (Kadota et al., 2005; Pardo et al., 2005).

OPN has been found colocalized with MMP-7 in alveolar epithelial cells of IPF lungs, and microarray studies have shown significant interaction between OPN and MMP-7 (Pardo et al., 2005). Positive feedback mechanisms have been previously proposed for OPN and MMP-2, OPN and MMP-3 (Agnihotri et al., 2001; Gao et al., 2004; Philip & Kundu, 2003). In the same way, MMP-7 cleaves and activates OPN, and is induced by OPN (Agnihotri et al., 2001; Pardo et al., 2005). Thus, this proposed positive self-perpetuating loop could facilitate pulmonary fibrosis, a chronic irreversible lung disease.

The pivotal role of MMP-7 and OPN in pulmonary fibrosis and the interaction between MMP-7 and OPN in the pathogenesis of IPF have been demonstrated. However, to date, there has been no study on the association between MMP-7 and OPN gene polymorphisms and CWP risk, as well as their roles in CWP. Considering that CWP shares some common characteristics with IPF, we hypothesize that genetic variations in MMP-7 and OPN may play potential roles in CWP. In this study, we evaluated the frequency distribution of four common single nucleotide polymorphisms (SNPs, rs10502001 in MMP-7, rs11728697, rs1126772, rs9138 in OPN) within two related genes in CWP patients and control individuals in a Chinese population to investigate the associations of these SNPs with the susceptibility to CWP. This information would contribute in understanding the underlying genetic mechanisms leading to CWP.

**Materials and methods**

**Study subjects**

In this study, a total of 1391 males were recruited from the coal mines of Xuzhou Mining Business Group Co., Ltd. between January 2006 and December 2010, as described previously (Wang et al., 2010). These subjects include 697 CWP patients and 694 controls. Subjects were excluded if they had clinical evidence of autoimmunity diseases, had received immunosuppressive or immunostimulatory therapy, or were subjected to radiotherapy. To confirm diagnoses, high kilovolt chest X-ray and physical examinations were performed based on the China National Diagnostic Criteria for Pneumoconiosis (GBZ 70-2002), which are the same as that of the 1980 International Labour Organization in the judgment of opacity profusion (Wang et al., 2011). The pneumoconiosis cases were classified into stages I, II or III according to the size, profusion and distribution range of opacities. The chest X-rays were assessed by two independent physicians. The controls were selected from the same coal mines, who were matched for age (within 5 years), dust exposure period and job type. Using a double-blind method, epidemiological questionnaire was conducted by trained interviewers. Individual information was collected including age, respiratory symptoms, occupational histories, smoking habits and other risk factors for the disease. After interview, about 5 ml of blood sample was collected from each subject and used for routine laboratory tests. Prior to their enrollment in this study, written informed consents were obtained from all subjects. The study protocol was approved by the Institutional Review Board of Nanjing Medical University.

**SNPs selection**

To select the most likely functional SNPs influencing MMP-7 and OPN gene, we chose all the non-synonymous SNPs and the SNPs located in 3’ and 5’UTR, based on the UCSC Genome browser (February 2009 [GRCh37/hg19] assembly; Common SNPs [138]). We included the following criteria for SNPs: (i) the SNPs should be located in the exon, 3’UTR or 5’UTR, (ii) the SNPs should be non-synonymous, (iii) the minor allele frequency (MAF) should be >5% in the Chinese Han Beijing (CHB) population and (iii) in the case of multiple SNPs in the same haplotype block (linkage coefficient $r^2$>0.8 in CHB of 1000 Genome database), only the most representative SNP was selected. Ultimately, three SNPs (rs11728697, rs1126772 and rs9138) in OPN, one SNP rs10502001 in MMP-7 were included in the study. Among them, rs11728697 and rs10502001 are located in the exon; the other two are in 3’UTR.

**Genotyping**

The genomic DNA from peripheral blood lymphocytes was extracted by the conventional phenol-chloroform method. Samples from patients and control subjects were genotyped for the two polymorphic sites using TaqMan allelic discrimination assay with the 7900 HT Real Time PCR System (Applied Biosystem, Foster City, CA). The fluorescence intensity of each well in the TaqMan assay plate was read and the fluorescence data files from each plate were analyzed using automated allele-calling software SDS 2.4 (Applied Biosystems, Foster City, CA).

The sequences of the primer and probe for each SNP are available on request (BioSteed BioTechnologies Co., Ltd., Nanjing, China). Further detailed information about the sequence of each primer and probe is listed in the supplemental file. Amplification was performed in a total volume of 5 µl, containing 50 ng of genomic DNA, 2.5 µl of Mix, 0.25 µl of each primer, 0.125 µl of each probe and 1.25 µl Nuclease-Free Water, under the following conditions: 50 °C for 2 min and 95 °C for 10 min followed by 45 cycles at 95 °C for 15 s and 60°C for 1 min. Negative controls were included in each
plate to ensure accuracy of the genotyping. Genotyping was conducted by two researchers independently in a blinded fashion without knowledge of the workers’ personal details or case status. We assessed genotype data quality by typing >10% random replicate samples and the concordance rate was 100.0%.

Statistical analyses

Student’s t-test (for continuous variables) or χ²-test (for categorical variables) were used to evaluate the distribution differences of demographic characteristics, selected variables and frequencies of genotypes of SNPs between the CWP cases and controls. For the case-control study, the Hardy–Weinberg equilibrium (HWE) for genotype distribution in controls was tested by a goodness-of-fit χ² test. Crude and processed odds ratios (ORs) adjusted for possible confounders (age, exposure years, pack-years smoked and occupation) and their 95% confidence intervals (CIs) were calculated to evaluate the strength of associations between the polymorphisms in MMP-7 as well as OPN and risk of CWP under various genetic models by multivariate unconditional logistic regression analyses. These were defined as Aa versus AA and aa versus AA for co-dominant, aa + Aa versus AA for dominant, aa versus AA + Aa for recessive, and a versus A for additive model (A: major allele; a: minor allele). In this study, the age and dust-exposure cutoff used for the stratified analysis were based on the median of ages and dust-exposure years of the recruited patients and controls. The statistical power was calculated by using PS software (http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize). Further Bonferroni’s correction for multiple testing was used; considering that four genetic models were tested for four polymorphisms when assessing relationship between CWP risk and SNPs, \( p < 0.003 \) (0.05/16) indicated statistical significant results. In all other cases, \( p < 0.05 \) was considered as statistically significant. All statistical tests were two sided. Statistical analyses were done using SPSS software (version 20.0).

Results

In this case-control study, the frequency distributions of selected characteristics between cases and controls are presented in Table 1. Briefly, there was no significant difference in the distribution of age \( (p = 0.103) \), exposure years \( (p = 0.105) \) and work types \( (p = 0.534) \) between the cases and controls. The smoking status of CWP was similar to the controls \( (p = 0.250) \), but the pack-years smoked in CWP cases was significantly less than that in controls \( (p < 0.001) \). In addition, the stages from I to III of the cases were 59.5, 31.4 and 9.0%, respectively.

The primary information and allele frequencies observed are listed in Table 2. According to HWE, the observed genotype frequency of the studied polymorphisms in control subjects did not show significant deviation from expected frequency. The MAF of all the four SNPs was consistent with that reported in the NCBI dbSNP (http://www.ncbi.nlm.nih.gov/snp) based on HapMap-HCB.

The ORs and \( p \) values calculated by multivariate logistic regression analysis under different genetic models to assess the effect of each SNP on CWP risk (adjusting for age, exposure years, pack-years smoked and occupation) are shown in Table 3. The distribution of genotypes of the selected four SNPs in CWP patients and controls are also listed. No significant difference in genotype frequencies of \( OPN \) rs9138 was observed between CWP patients and healthy controls under any of the genetic models. The other three SNPs (MMP-7 rs10502001, \( OPN \) rs1126772 and \( OPN \) rs11728697) were associated with CWP risk under at least one genetic model. Under the recessive model (GG versus AA + AG; adjusted OR = 2.09, 95% CI = 1.17–3.72, \( p = 0.013 \)) of \( OPN \) rs1126772, an aberrant distribution of genotypes was found in CWP patients compared with healthy controls. In addition, the risk alleles of MMP-7 rs10502001 and \( OPN \) rs11728697 both increased the susceptibility to CWP under cocdominant (TT versus CC, adjusted OR = 2.05, 95% CI = 1.38–3.05, \( p < 0.001 \) for MMP-7 rs10502001; CT versus CC, adjusted OR = 1.32, 95% CI = 1.05–1.66, \( p = 0.019 \) for MMP-7 rs10502001; TT versus CC, adjusted OR = 1.66, 95% CI = 1.04–2.44, \( p = 0.009 \) for \( OPN \) rs11728697), recessive (TT versus CC+CT, adjusted OR = 1.80, 95% CI = 1.01–3.20, \( p = 0.045 \) for MMP-7 rs10502001; TT versus CC+CT, adjusted OR = 2.48, 95% CI = 1.37–4.51, \( p = 0.003 \) for \( OPN \) rs11728697), and additive (T versus C, adjusted OR = 1.31, 95% CI = 1.03–1.67, \( p = 0.029 \) for MMP-7 rs10502001; T versus C, adjusted OR = 1.35, 95% CI = 1.06–1.72, \( p = 0.016 \) for \( OPN \) rs11728697) models. However, only the polymorphism MMP-7 rs10502001 was still associated with an increased risk of CWP under codominant model even after Bonferroni correction.

The associations between each of three SNPs (MMP-7 rs10502001, \( OPN \) rs11728697, \( OPN \) rs1126772) and CWP risk were further observed by stratification analysis based on exposure years and pack-years smoked under a recessive model. As shown in Table 4, individuals with the MMP-7 rs10502001 TT genotype had an increased risk of CWP than

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Table 1. Demographic and selected variables among the CWP cases and control subjects.

| Variables                  | CWP cases (n = 697) | Controls (n = 694) | p       |
|----------------------------|--------------------|--------------------|---------|
| Age, years (mean ± SD)     | 68.0 ± 11.1        | 67.1 ± 8.4         | 0.103   |
| Exposure years (mean ± SD) | 26.6 ± 9.0         | 27.3 ± 7.8         | 0.105   |
| Smoking status             |                    |                    | 0.250   |
| Never                      | 340 48.8           | 360 51.9           |         |
| Ever                       | 357 51.2           | 334 48.1           |         |
| Former                     | 163 23.4           | 91 13.1            |         |
| Current                    | 194 27.8           | 243 35.0           |         |
| Pack-years smoked          |                    |                    |         |
| 0                          | 340 49.2           | 360 52.6           | <0.001  |
| ~20                        | 223 32.0           | 132 19.0           |         |
| >20                        | 134 19.2           | 202 29.1           |         |
| Job type                   |                    |                    | 0.534   |
| Tunnel and coal mining     | 663 95.1           | 652 94.0           |         |
| Transport                  | 16 2.3             | 17 2.5             |         |
| Others                     | 18 2.6             | 25 3.6             |         |
| Stage                      |                    |                    |         |
| I                          | 415 59.5           |                    |         |
| II                         | 219 31.4           |                    |         |
| III                        | 63 9.0             |                    |         |
those with the CC/CT genotypes, and this increased risk was more evident among the subgroup of dust exposure years ≤27 (adjusted OR = 2.57, 95% CI = 1.44–4.60, p = 0.001) and smokers with pack-years smoked ≤20 (adjusted OR = 2.87, 95% CI = 1.06–7.76, p = 0.037), as well as nonsmokers (adjusted OR = 1.93, 95% CI = 1.11–3.33, p = 0.019). In addition, the variant of \( OPN \) rs1126772 also increased CWP risk of smokers with pack-years smoked ≤20 (adjusted OR = 2.45, 95% CI = 1.03–5.84, p = 0.043). Besides, association was observed between the \( OPN \) rs1126772 polymorphism and subjects with exposure years ≥27 (adjusted OR = 1.99, 95% CI = 1.17–3.40, p = 0.012). The function prediction results performed by Regulome DB (http://www.regulomedb.org/) are listed in Table 5; however, there is no data about \( OPN \) rs1126772.

Table 2. Primary information of genotyped SNPs.

| Gene | Cluster ID | Region | dbSNP allele | Function | Protein residue | MAF Case | MAF Control | HWE |
|------|------------|--------|--------------|----------|----------------|----------|-------------|-----|
| MMP-7 | rs10502001 | Exon-2 | C>T | missense | Arg/His | 0.31 | 0.24 | 0.167 |
| \( OPN \) | rs1126772 | 3’UTR | A>G | – | – | 0.28 | 0.26 | 0.367 |
| \( OPN \) | rs11728697 | Exon-4 | C>T | missense | Ala/Val | 0.34 | 0.30 | 0.354 |
| \( OPN \) | rs9138 | 3’UTR | C>A | – | – | 0.28 | 0.30 | 0.854 |

HWE, Hardy–Weinberg equilibrium; p value in the control group.

Table 3. Distributions of genotypes and their associations with risk of CWP.

| Variables | CWP cases | | | Controls | | | |
|-----------|-----------|-------|-----------|-----------|-------|-------|
| \( MMP-7 \) rs10502001 | n = 684 | | n = 663 | | OR (95%CI) | p | OR (95%CI) | p |
| CC | 339 | 49.6 | 389 | 58.7 | 1.00 | – | 1.00 | – |
| CT | 264 | 38.6 | 229 | 34.5 | 1.32 (1.05–1.66) | 0.017 | 1.32 (1.05–1.66) | 0.019 |
| TT | 81 | 11.8 | 45 | 6.8 | 2.07 (1.40–3.06) | <0.001 | 2.05 (1.38–3.05) | <0.001* |
| Dominant | | | | | 1.45 (1.17–1.79) | <0.001 | 1.32 (0.96–1.80) | 0.088 |
| Recessive | | | | | 1.85 (1.26–2.70) | 0.002 | 1.80 (1.01–3.20) | 0.045 |
| Additive | | | | | 1.39 (1.18–1.64) | <0.001 | 1.31 (1.03–1.67) | 0.029 |
| \( OPN \) rs1126772 | n = 673 | | n = 661 | | OR (95%CI)* | p | OR (95%CI) | p |
| AA | 371 | 55.1 | 353 | 53.4 | 1.00 | – | 1.00 | – |
| AG | 230 | 34.2 | 267 | 40.4 | 0.82 (0.65–1.03) | 0.088 | 0.83 (0.66–1.04) | 0.108 |
| GG | 72 | 10.7 | 41 | 6.2 | 1.19 (0.77–1.82) | 0.433 | 1.30 (0.70–2.40) | 0.412 |
| Dominant | | | | | 0.93 (0.75–1.16) | 0.528 | 0.82 (0.60–1.12) | 0.213 |
| Recessive | | | | | 1.81 (1.22–2.70) | 0.004 | 2.09 (1.17–3.72) | 0.013 |
| Additive | | | | | 1.07 (0.91–1.26) | 0.433 | 1.03 (0.81–1.30) | 0.824 |
| \( OPN \) rs11728697 | n = 683 | | n = 669 | | OR (95%CI) | p | OR (95%CI) | p |
| CC | 305 | 44.7 | 327 | 48.9 | 1.00 | – | 1.00 | – |
| CT | 296 | 43.3 | 289 | 43.2 | 1.10 (0.88–1.38) | 0.415 | 1.11 (0.88–1.39) | 0.378 |
| TT | 82 | 12.0 | 53 | 7.9 | 1.66 (1.14–2.42) | 0.009 | 1.66 (1.04–2.44) | 0.009 |
| Dominant | | | | | 1.19 (0.96–1.47) | 0.120 | 1.25 (0.91–1.71) | 0.174 |
| Recessive | | | | | 1.59 (1.10–2.28) | 0.013 | 2.48 (1.37–4.51) | 0.003 |
| Additive | | | | | 1.21 (1.03–1.43) | 0.020 | 1.35 (1.06–1.72) | 0.016 |
| \( OPN \) rs9138 | n = 683 | | n = 655 | | OR (95%CI) | p | OR (95%CI) | p |
| CC | 363 | 53.1 | 316 | 48.2 | 1.00 | – | 1.00 | – |
| CA | 257 | 37.6 | 280 | 42.7 | 0.80 (0.64–1.00) | 0.052 | 0.80 (0.64–1.00) | 0.053 |
| AA | 63 | 9.2 | 59 | 9.0 | 0.93 (0.63–1.37) | 0.710 | 0.93 (0.63–1.37) | 0.710 |
| Dominant | | | | | 0.93 (0.66–1.02) | 0.073 | 1.01 (0.73–1.39) | 0.959 |
| Recessive | | | | | 1.03 (0.71–1.49) | 0.891 | 0.72 (0.43–1.20) | 0.206 |
| Additive | | | | | 0.90 (0.76–1.06) | 0.189 | 0.94 (0.74–1.19) | 0.595 |

- **Two-sided \( \chi^2 \) test.
- **Adjusted for age, exposure years, pack-years smoked and job type.
- *Statistically significant (p < 0.003) after Bonferroni correction.

Considering potential interactions of \( MMP-7 \) and \( OPN \) gene polymorphisms on risk of CWP, we combined these four polymorphisms based on the numbers of variant (risk) alleles (i.e. \( MMP-7 \) rs10502001 T, \( OPN \) rs1126772 G, \( OPN \) rs11728697 T, \( OPN \) rs9138 A alleles). As shown in Table 6, individuals with multiple risk alleles had a higher risk of CWP, compared with those with 0–1 risk allele, in a dose-dependent manner with increasing numbers of risk variant alleles conferring increasing risk (\( \chi^2 \) trend = 0.003). Specifically, individuals carrying 4–8 risk alleles had a significantly higher risk of CWP than those with 0–1 risk allele (adjusted OR = 1.75, 95% CI = 1.22–2.52, p = 0.002). Furthermore, logistic regression analysis was conducted to investigate the interaction between significant SNPs and smoking status. Nevertheless, no variant was interacted with...
MMP-7, with local inflammatory regulatory roles, can serve as a source of ECM molecules in pulmonary fibrosis. Third, myofibroblasts in the lungs, which are thought to be the main population of profibrotic fibroblasts (Tsukui et al., 2013). Several animal models have proved that OPN contributes to the collagen synthesis (López et al., 2013; Trueblood et al., 2001).

Table 4. Stratification analyses between the genotypes of three SNPs and CWP risk.

| Variables      | Cases | Controls | OR (95% CI) | p      | Cases | Controls | OR (95% CI) | p      | Cases | Controls | OR (95% CI) | p      |
|----------------|-------|----------|-------------|--------|-------|----------|-------------|--------|-------|----------|-------------|--------|
| Exposure years |       |          |             |        |       |          |             |        |       |          |             |        |
| <27            | 43/224| 18/240   | 2.57 (1.44–6.60) | 0.001 | 28/239| 16/244   | 1.78 (0.94–3.39) | 0.077 | 28/234| 19/240   | 1.49 (0.81–2.75) | 0.197 |
| ≥27            | 38/379| 27/378   | 1.47 (0.88–2.47) | 0.154 | 54/362| 37/372   | 1.48 (0.95–2.32) | 0.082 | 44/367| 22/380   | 1.99 (1.17–3.40) | 0.012 |
| Pack-years smoked |     |          |             |        |       |          |             |        |       |          |             |        |
| 0              | 39/295| 22/326   | 1.93 (1.11–3.33) | 0.019 | 40/295| 29/321   | 1.48 (0.89–2.46) | 0.128 | 32/298| 18/328   | 1.91 (1.05–3.48) | 0.638 |
| ~20            | 24/194| 5/121    | 2.87 (1.06–7.76) | 0.037 | 27/191| 7/118    | 2.45 (1.03–5.84) | 0.043 | 30/183| 11/111   | 1.61 (0.77–3.35) | 0.207 |
| >20            | 18/114| 17/171   | 1.49 (0.74–3.01) | 0.269 | 15/115| 17/177   | 1.40 (0.67–2.95) | 0.373 | 10/120| 12/181   | 1.19 (0.49–2.88) | 0.696 |

aVariant homozygote/Heterozygote + Wild type homozygote.
bAdjusted for age, exposure years, pack-years smoked and job type.

Discussion

MMP-7, which is involved in ECM natural turnover and wound healing, displays strong proteolytic activity against various kinds of ECM components such as collagen types IV, V, IX and X, proteoglycans, fibronectin, laminin, gelatin, elastin and entactin (Fujishima et al., 2010). Besides, it cleaves various non-ECM bioactive molecules including Fas ligand, pro-tumor necrosis factor α, E-cadherin, insulin-like growth factor-binding protein 3 and insulin-like growth factor binding protein 5 (Gearing et al., 1995; Hemers et al., 2005; McGuire et al., 2003; Miyamoto et al., 2004; Powell et al., 2001). Experimental study using MMP-7-deficient mice has demonstrated that MMP-7 is essential to epithelial cell migration over and re-epithelialization of the damaged airways (Dunsmore et al., 1998). In addition, MMP-7 is known to shed E-cadherin, which is necessary for the epithelial repair (McGuire et al., 2003). Therefore, MMP-7 seems to play a role in the formation of hyperplastic epithelial foci and disordered tissue repair in pulmonary fibrosis. Moreover, we can speculate other functions of MMP-7 in the pathophysiological events including inflammation and fibrosis (Morimoto et al., 2010). It contains the arginine-glycine-aspartate (RGD)-binding motif common to many ECM proteins (Oldberg et al., 1986), making it promote integrin- and CD44-mediated cell adhesion, migration and chemotaxis (Liaw et al., 1995; Marcondes et al., 2008). Potential mechanisms of its participating in pulmonary fibrosis can be summarized as the following aspects. First, OPN has well-described chemotactic properties for lymphocytes, neutrophils, and macrophages (Ashkar et al., 2000; Koh et al., 2007; O’Regan et al., 1999), thus involved in the recruitment and retention of these cells to sites of inflammation. In addition, classical mediators of acute inflammation (tumor necrosis factor-α, interleukin-1β), mainly secreted by inflammatory cells, could strongly induce OPN expression (Mazzali et al., 2002). This positive feedback effect further aggravates the inflammation of lung tissue. Second, although OPN has previously been shown to be a transforming growth factor-β1 (TGF-β1) response gene (Fagenholz et al., 2001), it may also function upstream of fibrogenic cytokine TGF-β1 by regulating its activation (Berman et al., 2004; Denhardt et al., 2001).

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Last, OPN could promote the proliferation, differentiation, migration and collagen synthesis of fibroblasts (Denhardt et al., 2001; Lenga et al., 2008; Pardo et al., 2005), that are essential for the expansion of their populations and the formation of fibroblastic foci, representing the “leading edge” of progressive fibrotic process (Crystal et al., 2002). And OPN has been suggested to serve as a useful marker of profibrotic fibroblasts (Tsukui et al., 2013). Several animal models have proved that OPN contributes to the collagen synthesis (López et al., 2013; Trueblood et al., 2001).

Table 5. Function prediction of SNPs.

| Gene | SNP_ID    | regolumeDB | eQTL | Dnase | TFBS |
|------|-----------|------------|------|-------|------|
| MMP-7| rs10502001| 4          | N    | Y     | Y    |
| OPN  | rs11728697| 1d         | Y    | Y     | Y    |
Table 6. Frequency distributions of the combined genotypes between CWP cases and controls.

| Risk Allele | CWP cases | | Controls | | | OR (95%CI) | p | OR (95%CI)* | p* |
|---|---|---|---|---|---|---|---|---|---|
| 0–1 | 150 | 23.22 | 170 | 28.29 | 1.00 | – | 1.00 | – | – |
| 2–3 | 375 | 58.05 | 353 | 58.74 | 1.20 (0.93–1.57) | 0.167 | 1.20 (0.92–1.56) | 0.180 | 0.83 (0.37–1.87) | 0.661 |
| 4–8 | 121 | 18.73 | 78 | 12.98 | 1.76 (1.23–2.52) | 0.002 | 1.75 (1.22–2.52) | 0.002 | – |
| $P_{	ext{trend}}$ | | | | | | | | | 0.003 |

*Adjusted for age, exposure years, pack-years smoked and job type.

Table 7. Interaction between MMP-7 rs10502001, OPN rs11728697, OPN rs1126772 and smoking on CWP risk: case-control analysis.

| Interaction markers | $\beta$ | OR (95% CI)* | p* |
|---|---|---|---|
| MMP-7 rs10502001 * smoking | –0.111 | 0.89 (0.42–1.92) | 0.776 |
| OPN rs11728697 * smoking | 0.145 | 1.16 (0.56–2.40) | 0.697 |
| OPN rs1126772 * smoking | –0.180 | 0.83 (0.37–1.87) | 0.661 |

*Adjusted for age, exposure years and job type in logistic regression model.

In vitro studies of tumor cells demonstrate OPN-dependent activation of MMPs (Desai et al., 2007; Rangaswami & Kundu, 2007), suggesting a link among MMPs and OPN. In human IPF lungs, OPN colocalized with MMP-7 in alveolar epithelial cells, and application of weakest link statistical models to microarray data suggested a significant interaction between OPN and MMP-7 (Pardo et al., 2005). Interestingly, MMP-7 and OPN are $\beta$-catenin target genes (Brabletz et al., 1999; El-Tanani et al., 2004). Recently, Chilosi et al. (2003) reported impressive activation of WNT/$\beta$-catenin in IPF lungs. OPN and MMP-7 may be induced by aberrant activation of the WNT/$\beta$-catenin pathway, and each affects the function and expression of the other gene, thus representing a local positive feedback mechanism that facilitates a chronic, relentless lung disease.

As far as we know, this is the first report of genetic susceptibility of CWP due to MMP-7 and OPN gene polymorphisms in Chinese. In the present study, MMP-7 rs10502001 and OPN rs11728697 have robust associations with CWP risk. Genetic mutations in the exonic region, causing amino acid changes, may lead to possible variation of proteins' properties including structure and function. Thus, polymorphisms of MMP-7 rs10502001 and OPN rs11728697 could consequently alter features of corresponding proteins, making them more conducive to promoting the development of pulmonary fibrosis. Unfortunately, the impacts of these two variants on the characteristics of corresponding proteins have not been investigated yet. However, functional prediction results show that MMP-7 rs10502001 is a locus of Transcription Factor-Binding Site (TFBS). And OPN rs11728697 is a locus of expression for Quantitative Trait Loci (eQTL) as well as TFBS. Therefore, it is possible that these two SNPs could affect transcription activity of corresponding genes, consequently making individuals more susceptible to CWP, though the exact molecular mechanisms have not been clear. Further, these risk variants might be in linkage disequilibrium with multiple biologically active variants, which interact with each other, epigenetic factors and the environment to increase the risk of CWP. However, these hypotheses need to be confirmed in further investigations. Several studies have demonstrated the associations of MMP7 rs10502001 and OPN rs11728697 with various lung diseases. For instance, Kastelijn et al. (2010) discovered that the TT genotype of MMP-7 rs10502001 was a hazardous genotype for bronchiolitis obliterans syndrome after lung transplantation. Consistently, polymorphisms of MMP-7 and OPN indeed have important roles in the susceptibility to diseases.

Interestingly, we found that the deleterious effects of MMP-7 rs10502001 TT genotype were more pronounced in nonsmokers or mild to moderate smokers with long dust exposure history. In addition, we also found the association between OPN rs11728697 and the susceptibility to CWP was more remarkable among mild to moderate smokers. However, several studies have addressed smoking can induce lung fibrosis through a variety of mechanisms (Franks & Galvin, 2015; Katzenstein, 2014). Although the reasons hiding behind are unknown, it is possible that individuals in those subgroups more likely were exposed to more risk factors involved in the etiology of CWP risk.

Although we determined the relationship between MMP-7 as well as OPN SNPs and CWP risk, our study still exhibits several limitations, including moderate sample size, especially for subgroup and interaction analyses, and retrospective design. Results from the study require confirmation through well-designed, larger prospective studies. However, all the subjects were collected from annually routine physical examination, which may reduce the selection bias and make the results more accurate. Our results also need validation among different ethnic populations because some genetic markers are ethnic specific.

Conclusions

In conclusion, our results show associations between the genetic polymorphisms in MMP-7 (rs10502001) and OPN (rs11728697, rs1126772) and CWP risk in a Chinese population. Further validation studies and functional researches are warranted to validate our findings and elucidate the exact mechanisms by which MMP-7 and OPN variants contribute to the susceptibility of these individuals to CWP.

Declaration of interest

The authors declare that they have no competing interests.

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