SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
PREDICTIVE MARKERS
RISK FACTORS
GENETICS RESEARCH
RESPIRATORY TRACT DISEASES

Received
10 April 2013

Accepted
27 August 2013

Published
2 October 2013

Correspondence and requests for materials should be addressed to H.S. (huahaoshen@163.com)

Genetic Polymorphism of Matrix Metalloproteinase Family and Chronic Obstructive Pulmonary Disease Susceptibility: a Meta-analysis

Hongbin Zhou, Yinfang Wu, Yan Jin, Jiesen Zhou, Chao Zhang, Luanqing Che, Jiyong Jing, Zhihua Chen, Wen Li & Huahao Shen

Department of Respiratory and Critical Care Medicine, Second Hospital of Zhejiang University School of Medicine, Hangzhou, Zhejiang, China.

Matrix metalloproteinase (MMP) family is considered to be associated with chronic obstructive pulmonary disease (COPD) pathogenesis, however, no consistent results have been provided by previous studies. In this report, we performed Meta analysis to investigate the association between four kinds of MMP single nucleotide polymorphisms (SNP, MMP1 -1607 1G/2G, MMP3 -1171 5A/6A, MMP9 -1562 C/T, MMP12 -82 A/G) and COPD risk from 21 studies including 4184 cases and 5716 controls. Both overall and subgroup association between SNP and COPD susceptibility were tested. There was no evident association between MMP polymorphisms and COPD susceptibility in general population. On the other hand, subgroup analysis suggested that MMP9 -1562 C/T polymorphism was related to COPD, as we found that C allele carriers were at lower risk in some subgroups stratified by lung function, age and genotype identification method, compared with TT homozygotes. Our results indicated the genotype TT might be one genetic risk factor of severe COPD.

Chronic obstructive pulmonary disease (COPD) is a chronic airway inflammatory disease, which is characterized by not fully reversible airflow limitation, inflammatory cells infiltration, mucus overproduction and airway remodeling[1]. As a complex disease, the precise molecular mechanism of COPD is still unknown. However, it was widely accepted that the occurrence of COPD relied on the interaction of gene and environment.

Protease and anti-protease imbalance was considered as an important mechanism involved in the pathogenesis of COPD. Since the discovery of relationship between a α 1-antitrypsin and COPD, no other proteases or anti-proteases have been confirmed to be associated with this disease[2]. Matrix metalloproteinase (MMP) is a group of protease, which mediate various physiological and pathological processes. So far, at least 24 kinds of MMPs have been identified in human[3]. Increasing evidence from animal experiments suggested that MMPs played a pivotal role in COPD[4–11].

The activity of MMPs is dependent on the gene encoding them. The existence of gene polymorphism determines the different expression level of these genes among individuals, which ultimately result in different phenotype of disease in a population. In the past decade, considerable efforts have been made to find out the relationship between MMP single nucleotide polymorphism (SNP) and COPD risk in several populations[12–32]. However, the results of different researches were not consistent. Some reports showed that specific MMP genotype was related to occurrence of COPD[13,14,19,23,27,31,32], while other reports did not support the association between MMP polymorphism and COPD susceptibility[12,15–18,20–22,24–26,28–30]. These contradictory findings may be partly due to limited sample size, false-positive results and publication bias. In order to identify which MMP polymorphism play the key role in COPD occurrence, we conducted a comprehensive meta-analysis to quantify the overall risk of MMP polymorphisms on COPD.

Results

Characteristics of included studies. We identified 123 related articles, of which 30 studies were potentially appropriate. 7 studies did not examine any MMP single nucleotide polymorphism (SNP) mentioned above. 3

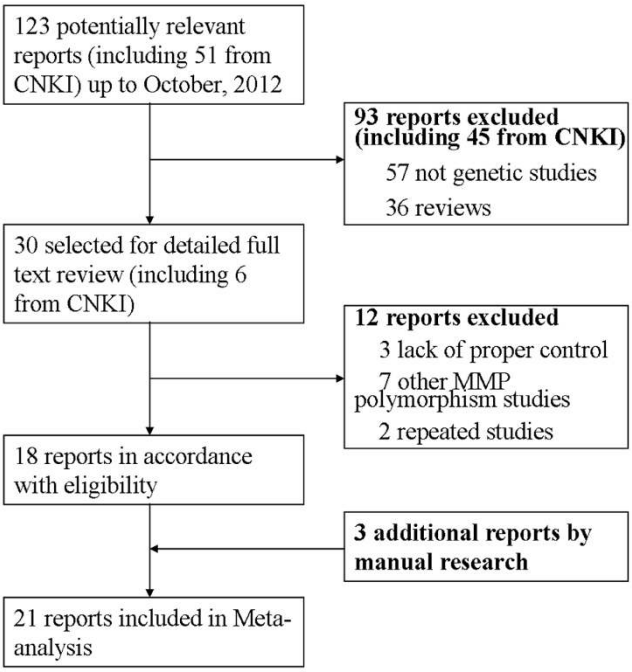

Figure 1 | Study identification, inclusion, and exclusion for meta-analysis.

studies were excluded because of lack of proper control. Further more, 2 repeated studies were also ruled out. Finally, 3 additional studies by manual search were added to analysis. Thus, 21 studies including 4184 cases and 5716 controls met the including criteria (Figure 1). MMP1 SNP and MMP9 SNP were both mentioned in 12 studies, while 5 and 6 studies provided the association of MMP3 and MMP12 SNPs with COPD, respectively. The study characteristics were listed in Table 1. COPD patients were diagnosed through lung function index in all studies expect two by Enewold *et al.*[15] and Minematsu *et al.*[24], respectively. Population and hospital based controls were involved in different studies. In addition, frequency-matched controls to the cases by ethnicity, sex, age and smoking status were applied in some studies. A classical polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) assay was performed in 15 of the 21studies.

Overall effects for alleles and genotypes. The distribution of alleles and genotypes of each study was listed in Table 2. Due to existence of moderate to high heterogeneity among studies, the random effect model was used to evaluate the odds ratio. For all the four MMP polymorphisms, we did not find the distribution of alleles was related to COPD risk (i.e. MMP1 1G versus 2G: OR 0.99, 95% CI 0.89–1.10, $p = 0.81$) (Table 3). On the other hand, we did not observe a significant association between genotypes and COPD risk through a series of comparisons, including dominant model (i.e. MMP9 CC versus CT + TT: OR 0.80, 95% CI 0.57–1.14, $p = 0.22$), recessive model (i.e. MMP9 CC + CT versus TT: OR 0.75, 95% CI 0.45–1,24, $p = 0.26$), and homozygote model (i.e. MMP9 CC versus TT: OR 0.71, 95% CI 0.40–1.24, $p = 0.23$) (Table 3).

Subgroup analysis. To exclude the effect of confound factors, such as genetic background, smoking index, lung function, detection methods etc., subgroup analysis was introduced to further elucidate the relationship between MMP SNPs and the risk of COPD. We divided included studies into 2 to 3 subgroups according to ethnicity (Asian or Caucasian), smoking index

Table 1 | Summary of 21 studies investigating association between MMP polymorphisms and COPD risk

author	year	ethnicity	sample size		age matched	smoking index matched	lung function of COPD		COPD diagnosis	genotype identification
			case	control			FEV1/FVC%	FEV1/pre%		
Cai	2010	Asian	80	90	Yes	Yes	not mentioned	not mentioned	CMA guideline #	PCR-RFLP
Cheng	2009	Asian	184	212	Yes	Yes	45.8 ± 8.6	47.2 ± 16.3	ATS	PCR-RFLP
Diemen #	2011	Caucasian	178	1117	Yes	Yes	not mentioned	not mentioned	GOLD	Taqman
Enewold	2012	African	44	147	Yes	No	not mentioned	not mentioned	not mentioned	MassARRAY
		Caucasian	123	191	Yes	No				iPLEXTM platform
Han	2006	Asian	60	52	Yes	Yes	not mentioned	45.2 ± 12.6	CMA guideline #	PCR-RFLP
Haq	2010	Caucasian	977	876	No	No	47.4 ± 12.1	43.0 ± 15.1	GOLD	KASPar assay
Hersh	2005	Caucasian	304	441	Yes	No	41.5 ± 8.2	24.8 ± 6.5	NETT inclusion standard ##	Taqman
Hua	2010	Asian	180	180	Yes	Yes	not mentioned	50.3 ± 3.6	CMA guideline #	PCR-RFLP
Ito	2005	Asian	84	85	No	No	45.3 ± 9.8	44.9 ± 17.4	GOLD	PCR-RFLP
Korytina	2008	Caucasian	318	319	No	Yes	not mentioned	39.4 ± 17.8	GOLD	PCR-RFLP
Korytina	2012	Caucasian	391	514	No	No	58.7 ± 13.7	41.7 ± 19.3	GOLD	PCR-RFLP
Lee	2010	Asian	301	333	No	No	49.4 ± 13.1	63.0 ± 26.2	GOLD	ABI sequencer
Minematsu	2001	Asian	45	65	Yes	Yes	49 ± 17	not mentioned	LAA score on chest CT-scans, LAA > 8.0	PCR-RFLP
Santus	2009	Caucasian	147	133	Yes	No	not mentioned	50.3 ± 16	GOLD	ABI sequencer
Schirmer	2009	Caucasian	111	101	No	Unknown	not mentioned	not mentioned	GOLD	PCR-RFLP
Sun	2005	Asian	59	109	Yes	Yes	not mentioned	not mentioned	CMA guideline #	PCR-RFLP
Sun	2012	Asian	80	74	Yes	Yes	47.28 ± 10.09	41.29 ± 15.59	CMA guideline #	PCR-RFLP
Tesfaigzi	2006	Caucasian*	123	262	No	No	not mentioned	58.6 (19–99)**	GOLD	PCR-RFLP
Zhang	2004	Asian	148	197	Yes	Yes	52.44 ± 10.77	52.3 ± 18.24	CMA guideline #	PCR-RFLP
Zhang	2005	Asian	147	120	No	Yes	53 ± 11	53 ± 18	CMA guideline #	PCR-RFLP
Zhou	2004	Asian	100	98	Yes	Yes	54.56 ± 9.85	63.14 ± 17.37	GOLD	PCR-RFLP

*The people recruited in this study consist of non-Hispanic white, Hispanic and others.
**the data were shown as average (min - max).
#The guideline was published by Chinese Medical Association (CMA) for diagnosis and treatment of COPD in 2002.
##The cases were from NETT (National Emphysema Treatment Trial) according to standard as follows: 1. FEV1 < 45% prediction, evidence of hyperinflation on pulmonary function testing; 2. Bilateral emphysema confirmed by HRCT.

Table 2 | the distribution of alleles and genotypes of MMPs in related studies

		Allele				Genotype						
		case		control		case			control			HWE (p)
MMP1 -1607 1G/2G (rs1799750)		**1G**	**2G**	**1G**	**2G**	**1G1G**	**1G2G**	**2G2G**	**1G1G**	**1G2G**	**2G2G**	
Cai	2010	34	126	56	124	6	22	52	15	26	49	0.002
Cheng	2009	121	247	104	320	20	81	63	16	72	124	0.229
Diemen	2011	182	174	1155	1079	44	94	40	295	565	257	0.669
Enewold	2012	177	157	352	324	51	75	41	92	168	78	0.938
Haq	2010	1006	948	911	841	273	460	244	228	455	193	0.231
Hersh	2005	298	310	424	458	73	152	79	102	220	119	0.987
Korytina	2008	271	365	261	377	65	141	112	51	159	109	0.580
Korytina	2012	450	332	464	304	138	174	79	150	164	70	0.036
Lee	2010	186	414	212	450	29	128	143	42	128	161	0.042
Tesfaigzi	2006	121	123	259	255	32	57	33	70	119	68	0.236
Sun	2005	32	86	40	178	5	22	32	9	22	78	0.001
Zhang	2005	92	202	102	138	15	62	70	31	40	49	<0.001
MMP3 -1171 5A/6A (rs35068180)		**5A**	**6A**	**5A**	**6A**	**5A5A**	**5A6A**	**6A6A**	**5A5A**	**5A6A**	**6A6A**	
Cheng	2009	342	26	401	23	158	26	0	189	23	0	0.404
Korytina	2012	22	780	46	664	0	22	369	0	46	309	0.192
Santus	2009	140	154	131	135	25	90	32	36	59	38	0.194
Schirmer	2009	89	93	88	110	26	37	28	23	42	34	0.161
Sun	2012	84	76	67	81	26	32	22	19	26	29	0.072
MMP9 -1562 C/T (rs3918242)		**C**	**T**	**C**	**T**	**CC**	**CT**	**TT**	**CC**	**CT**	**TT**	
Cheng	2009	233	135	320	104	76	81	27	124	72	16	0.229
Han	2006	76	44	72	32	25	26	9	26	20	6	0.483
Hua	2010	300	60	340	20	120	60	0	162	16	2	0.040
Ito	2005	145	23	144	26	63	19	2	60	24	1	0.408
Korytina	2008	560	76	556	82	248	64	6	241	74	4	0.523
Korytina	2012	685	97	758	110	300	85	6	330	98	6	0.674
Lee	2010	527	61	533	99	234	59	1	226	81	9	0.596
Minematsu	2001	68	22	114	16	25	18	2	50	14	1	0.986
Schirmer	2009	162	16	178	16	74	14	1	81	16	0	0.376
Tesfaigzi	2006	195	43	439	67	82	31	6	192	55	6	0.392
Zhang	2005	253	41	215	25	106	41	0	98	19	3	0.097
Zhou	2004	194	2	186	14	96	2	0	86	14	0	0.452
MMP12 -82 A/G (rs2276109)		**A**	**G**	**A**	**G**	**AA**	**AG**	**GG**	**AA**	**AG**	**GG**	
Diemen	2011	308	56	1905	345	130	48	4	812	281	32	0.202
Haq	2010	1749	205	1524	228	782	185	10	657	210	9	0.082
Korytina	2008	567	69	579	59	249	69	0	260	59	0	0.069
Korytina	2012	700	82	787	85	309	82	0	353	81	2	0.243
Schirmer	2009	194	28	186	16	84	26	1	85	16	0	0.387
Zhang	2004	289	7	386	8	141	7	0	189	8	0	0.877

(matched or not), age (matched or not), lung function (FEV_1/prediction%) and genotype detection method (RFLP or not).

For the MMP9 -1562C/T polymorphism, ethnicity and smoking index did not affect the distribution of alleles and genotypes between COPD patients and controls. Although age did not affect the distribution of alleles in these two subpopulations, it did affect genotypes distribution, especially for TT genotype. In age matched subgroup, carriers of C allele had much lower susceptibility of COPD, compared with TT homozygotes (CC + CT vs. TT: OR 0.56, 95% CI 0.33–0.96, p = 0.03; CC vs. TT: OR 0.44, 95% CI 0.25–0.77, p = 0.004) (Figure 2a–b). Just like age, lung function also affected TT genotype distribution in two subpopulations. In one subgroup where COPD patients with relatively worse lung function (FEV1 < 50% prediction), carriers of C allele were at much lower risk of COPD, compared with TT homozygotes (CC + CT vs. TT: OR 0.60, 95% CI 0.37–0.99, p = 0.05; CC vs. TT: OR 0.53, 95% CI 0.31–0.88, p = 0.01) (Figure 2 c–d). The method of genotype identification seemed to be another factor that related to the genotype distribution in the two subpopulations. The risk of COPD was lower among carriers of C allele than that among TT homozygotes in the subgroup where RFLP was used to detect genotype (CC + CT vs. TT: OR, 0.61, 95% CI 0.41–0.92, p = 0.02; CC vs. TT: OR0.54, 95% CI 0.35–0.82, p = 0.004) (Figure 2 e–f).

For the MMP1 -1607 1G/2G polymorphism, all the factors mentioned above seemed not to be associated with the distribution of both alleles and genotypes between COPD patients and controls (Supplementary Table S1).

For the MMP3 and MMP12 polymorphisms, we did not carry out subgroup analysis because of limited number of studies.

Heterogeneity analysis. For MMP9 -1562C/T polymorphism, I^2 showed a moderate variation under most comparisons when we performed overall and subgroup analysis. However, in the subgroup of Caucasian, there was almost no variation. Moreover, in the age matched, worse lung function and RFLP subgroup, no heterogeneity was detected except for the comparison between CC and CT + TT ($I^2 > 80\%$ in these subgroups) (Table 3, Supplementary Table S2).

For the overall and subgroup analysis of MMP1 -1607 1G/2G polymorphism, I^2 showed a moderate variation under most comparisons. In the subgroup of Caucasian and non-RFLP, no variation was found except for the comparison between 1G1G and 1G2G + 2G2G

Table 3 | Pooled odds ratio for COPD susceptibility, heterogeneity and publication bias in meta-anlysis: comparison of alleles and genotypes

Comparison	Study number	OR (95% CI)	P value	Heterogeneity		Publication bias	
				I^2	$P_{heterogeneity}$	Begg	Egger
MMP1 -1607 1G/2G (rs1799750)							
1G vs. 2G	12	0.99 (0.89, 1.10)	0.81	53%	0.01	0.945	0.980
1G1G + 1G2G vs. 2G2G	12	1.03 (0.86, 1.22)	0.76	58%	0.007	1	0.352
1G1G vs. 1G2G + 2G2G	12	0.99 (0.81, 1.21)	0.94	58%	0.006	0.244	0.274
1G1G vs. 2G2G	12	0.93 (0.77, 1.12)	0.44	38%	0.08	0.732	0.568
MMP3 -1171 5A/6A (rs35068180)							
5A vs. 6A	5	0.88 (0.61, 1.27)	0.50	71%	0.009	0.806	0.441
5A5A + 5A6A vs. 6A6A	4	0.92 (0.58, 1.46)	0.73	58%	0.07	0.089	0.100
5A5Avs. 5A6A + 6A6A	4	0.91 (0.58, 1.41)	0.66	50%	0.11	0.089	0.049
5A5A vs. 6A6A	3	1.18 (0.76, 1.82)	0.46	0%	0.42	0.296	0.210
MMP9 -1562 C/T (rs3918242)							
C vs. T	12	0.83 (0.62, 1.12)	0.22	79%	<0.0001	0.732	0.953
CC + CT vs. TT	11	0.75 (0.45, 1.24)	0.26	22%	0.24	0.533	0.100
CC vs. CT + TT	12	0.80 (0.57, 1.14)	0.22	81%	<0.0001	0.837	0.732
CC vs. TT	11	0.71 (0.40, 1.24)	0.23	31%	0.15	0.533	0.082
MMP12 -82 A/G (rs2276109)							
A vs. G	6	0.98 (0.80, 1.20)	0.82	44%	0.11	0.133	0.066
AA + AG vs. GG	4	1.14 (0.59, 2.20)	0.69	0%	0.70	1	0.862
AA vs. AG + GG	6	0.96 (0.76, 1.21)	0.72	52%	0.07	0.452	0.078
AA vs. GG	4	1.17 (0.61, 2.25)	0.64	0%	0.71	1	0.938

(I^2 = 29% in Caucasian subgroup and I^2 = 31% in non-RFLP subgroup, respectively). For the subgroup analysis of age and lung function, I^2 showed low or no heterogeneity under different comparisons in different subgroups (Table 3, Supplementary Table S1).

For overall analysis of MMP3 -1171 5A/6A and MMP12 -82A/G polymorphism, I^2 showed a low or moderate variation under all comparisons (Table 3).

Sensitivity analysis. Sensitivity analysis was performed to test the effect of a specific study on the overall results. There were six studies not exhibiting Hardy–Weinberg equilibrium (HWE) (5 for MMP1 -1607 1G/2G and 1 for MMP9 -1562 C/T). However, omitting these studies did not significantly alter the pooled OR value in both polymorphisms. Although the genotypes distribution of MMP9 -1562 C/T in the study reported by Lee *et al.* was not deviate from HWE, we found this study affected the general results obviously. If this study was excluded, the pooled OR decreased from 0.75 to 0.61 under the comparison of CC + CT vs. TT (95% CI: 0.41–0.92, p = 0.02) and from 0.71 to 0.54 under the comparison of CC vs. TT (95% CI: 0.35–0.82, p = 0.004). (Supplementary Figure S1)

Publication bias. Publication bias was detected by Begg's and Egger's test. These tests did not show significant results in almost all comparisons (Table 3). The shape of funnel plots did not reveal evidence of obvious asymmetry (Figure 3). These results indicated little publication bias.

Discussion

In order to seek out the genetic variants related to COPD, much effort has been made to explore the association between gene polymorphism via case-control study or cohort study. Recently, several genome-wide association studies (GWAS) have identified novel SNPs located in chromosomal loci 15q25 with genome-wide significance for association with COPD[33–35]. However, we have not found any data about the association between MMP and COPD based on GWAS.

Matrix metalloproteinase is a family of zinc-dependent endopeptidases degrading all the main protein components of the extracellular matrix and playing an essential role in tissue remodeling and repair associated with development and inflammation[36]. MMP-1, MMP3 and MMP-12 are located in close proximity on chromosome 11 while MMP-9 is located on chromosome 20 [3]. Many clinical studies suggested that MMPs were involved in COPD as concentration of MMPs in serum or induced sputum in patients was higher than that in non-COPD patients or healthy volunteers[37–40]. The degree of COPD severity is dependent on lung function. Joos *et al.* reported that MMP1 -1607 1G/2G polymorphism was associated with the rate of decline in lung function, and the haplotypes consisting of MMP1 -1607 1G/2G and MMP12 + 357Asn/Ser polymorphism were also related to lung function decline rate[41]. On the other hand, Hunninghake *et al.* found that the minor allele (G) of a functional variant in the promoter region of MMP12 was associated with a reduced risk of COPD in the NAS cohort consisting of initially healthy adult men and a cohort of smokers[42]. Variation in gene promoter region might cause the change of protein activity, which was considered to be associated with COPD susceptibility.

However, from the results of present analysis, we failed to find correlation between genotypes of various MMP family members and the risk of COPD. It was a negative result, but was in accordance with the results of majority studies included in this analysis. Although the remaining studies showed significant association between certain genotype and COPD susceptibility, it could not be ruled out the existence of false positive results due to the reasons as follows. First, some studies contained a small sample size, so the results might not stable enough. Second, different detection methods in different studies were the source of deviation, which affected overall results. Third, the positive results reported by some authors were contradictory. For example, Minematsu[24] and Cheng[13] reported that CC was a protective genotype, while Lee[23] and Zhou[32] suggested that CC was a susceptible genotype. Due to these paradoxical results, no significant overall effect could be obtained.

The great discrepancy among different studies indicated the existence of heterogeneity. In fact, moderate to high heterogeneity was detected in almost all comparisons. Subgroup analysis was introduced to further seek out the source of heterogeneity. For the analysis of MMP9 and MMP1 polymorphism, all the studies were divided into subgroups according to ethnicity, age, smoking index, lung function and methods for genotype identification. After stratification, the heterogeneity of at least one subgroup reduced or disappeared. It demonstrated that these factors were at least part source of heterogeneity. In addition, we also found in some subgroups, including age matched

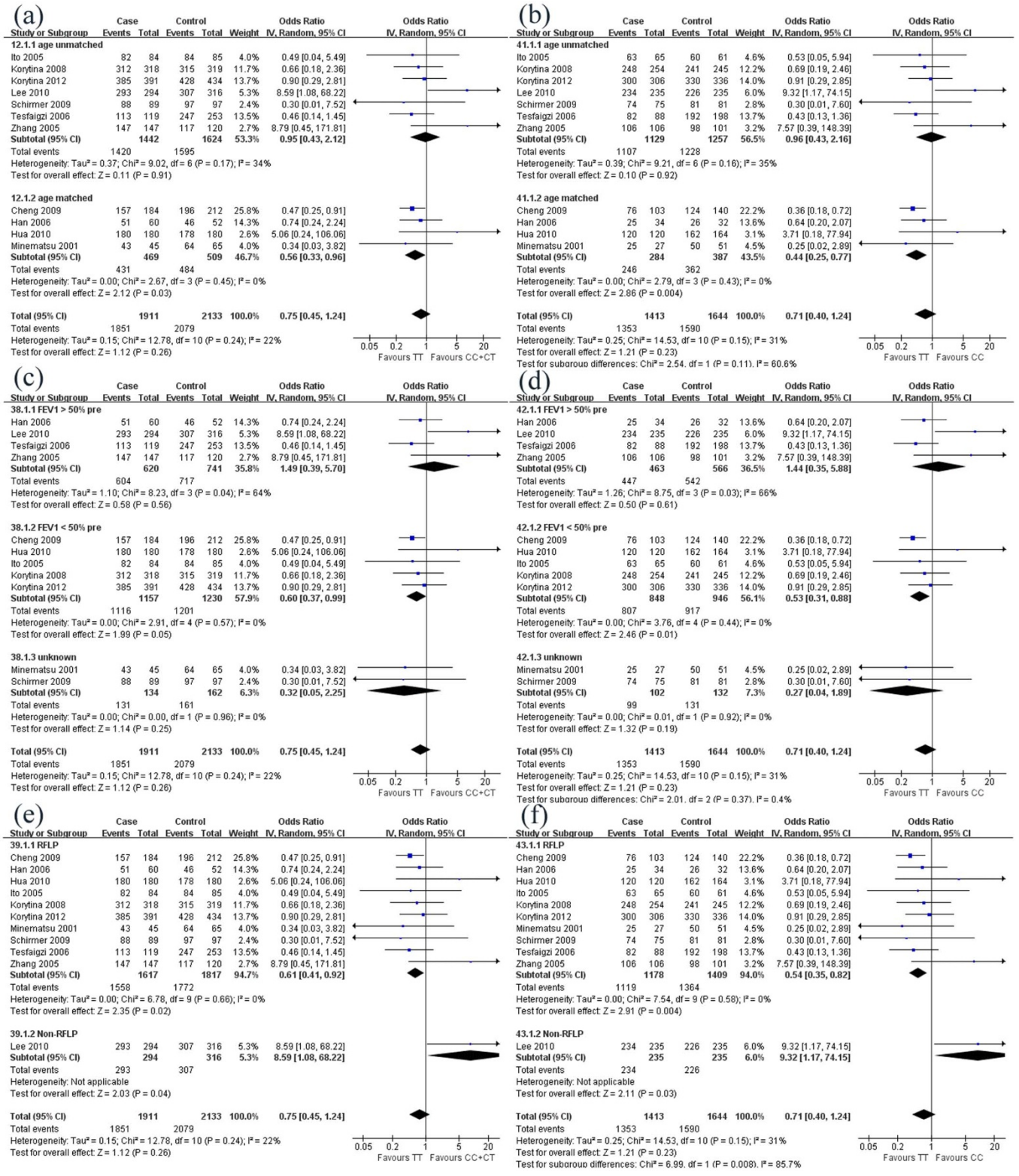

Figure 2 | Subgroup analysis of correlation between MMP9 polymorphism and COPD risk under two pairs of comparisons (CC + CT vs. TT; CC vs. TT). (a–b) The studies were divided into two groups according to age (unmatched or matched) under the comparison of CC + CT vs. TT (a) and CC vs. TT (b); (c–d) The studies were divided into three groups according to lung function (FEV1 > 50% prediction, FEV1 < 50% prediction or unknown) under the comparison of CC + CT vs. TT (c) and CC vs. TT (d); (e–f) The studies were divided into two groups according to genotype identification method (RFLP or Non-RFLP) under the comparison of CC + CT vs. TT (e) and CC vs. TT (f).

subgroup, worse lung function subgroup and RFLP subgroup, the carriers of allele C had much less risk of COPD. It was worth to note that the distribution of MMP9 genotypes in COPD patients with worse lung function is different from that in normal people and patients with better lung function. In fact, one report by Korytina (2008) *et al.*[21] showed that in very sever COPD patients (FEV_1 < 30% prediction) the allele T was more frequency. So it may provide us the direction to selecting proper subpopulation of COPD.

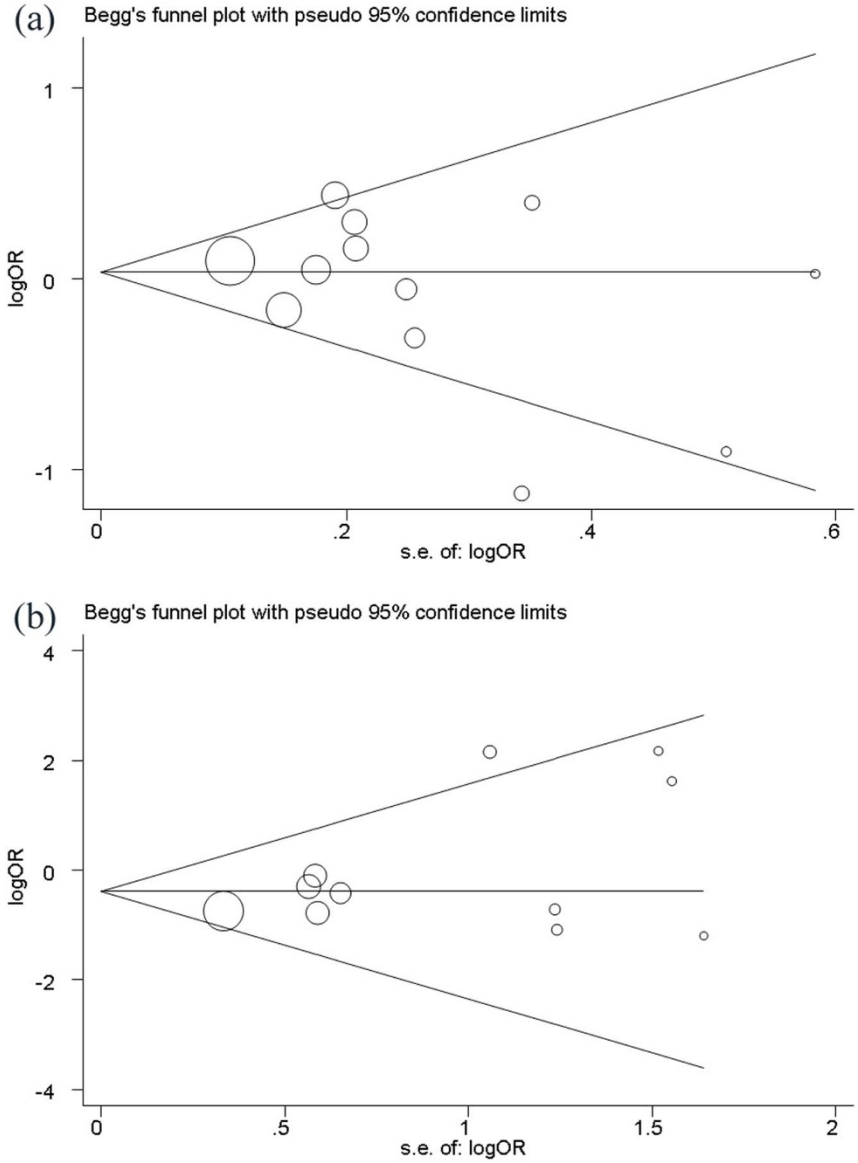

Figure 3 | Publication bias on MMP polymorphism. (a) Begg's funnel plot of the 12 eligible studies assessing MMP1 -1607 1G/2G polymorphism; (b) Begg's funnel plot of the 11 eligible studies assessing MMP9 -1567 C/T polymorphism.

Hardy–Weinberg equilibrium (HWE) results showed the p values of several studies were less than 0.05, which suggested the potential to influence the overall effect. Sensitivity analysis showed that these studies had minor effect on OR values, which indicated the stability of present work. Unexpectedly, another report greatly affected the main results. For MMP9 polymorphism, removing Lee's report would contribute to evident change of results. Apart from the inconsistency of some demography indexes between COPD cases and control subjects, this study used a unique method for genotype identification. It was the only study that did not use PCR-RFLP to analyze MMP9 genotype. This deficiency might partly explain the results mentioned above, but further research was needed to elucidate the discrepancy among these studies.

There were some limitations in the present analysis. First, the sample size was too small. The quantity of patients in these studies was just more than 4000. However, it was estimated that there were about 200 million COPD patients in the world. In other words, only a small part of patients were recruited to study. Second, the existence of confound factors severely affected the ultimate results. As discussed above, age, lung function, and even detection method were demonstrated to be confounders in present analysis. These confounders were present in almost every study we selected. Third, the data we obtained now were not comprehensive. Almost all the studies were either on Asian or Caucasian. African was one of the three largest ethnics on the earth, but so far we have found only one article on African. Moreover, the occurrence of COPD is not depend on one gene but a cluster of genes, so the gene linkage is important for exploring disease pathogenesis. However, we are lack of related data, which may prevent us from further research.

In conclusion, our present analyses did not show significant association between MMP SNPs and COPD risk in the whole population. However, we found TT genotype of MMP9 was a risk factor in the subpopulation with relatively worse lung function. So, it may be helpful to screen out potential severe COPD patients by detecting MMP9 genotype. In light of various deficiencies in present studies, there is a great need of further studies including large population, selecting appropriate control subjects, unifying detection method and paying much attention to gene linkage in order to confirm the role of MMP SNPs in the COPD susceptibility.

Methods

Search strategy. We carried out a comprehensive search strategy in various databases including Pubmed, Embase, Cochrane Library and China National Knowledge Infrastructure (CNKI) to seek out the articles which were about the association between MMP polymorphisms and the risk of COPD. The terms we used as follows: "chronic obstructive pulmonary disease", "COPD", "emphysema", "chronic bronchitis"; "matrix metalloproteinase", "interstitial collagenase", "stromelysin", "gelatinase", "macrophage elastase"; and "genetic polymorphism", "variant", "variation", "association". Additional studies were identified by a manual search from references of original studies or review articles on this topic. Only studies with full text articles published until October 2012 were included.

Study selection. The criteria for the papers selection were as follows: (1) studies with case-control or prospective longitudinal cohort design; (2) COPD as the outcome, with at least two comparison groups (COPD vs. healthy control groups); (3) the study including at least one of the four kinds of MMP polymorphisms (MMP1 -1607 1G/2G, MMP3 -1171 5A/6A, MMP9 -1562 C/T, MMP12 -82 A/G) in COPD cases and controls; (4) provide the available genotype frequency in COPD cases and healthy controls.

Data extraction. Information was carefully extracted from all eligible publications independently by two authors according to the inclusion criteria listed above. Once encountering disagreements, we resolved them by discussions with the third person. The data we extracted from papers contain basic information of study (author, publication year), population (sample size, ethnicity, age, lung function, and smoking index), COPD definition, genotype distribution in cases and controls, genotype identification method, Hardy-Weinberg equilibrium (HWE) test etc.

Data analysis. Odds ratio (OR) and 95% CIs were used to assess the strength of association between all kinds of MMP polymorphisms (MMP1 -1607 1G/2G, MMP3 -1171 5A/6A, MMP9 -1562 C/T, MMP12 -82 A/G) and COPD risk. Dominant, recessive and homozygote model were applied for genotype analysis.

Heterogeneity assumption was checked by the Cochran Q test. If p value for the Q test is over 0.10, we consider that there is lack of heterogeneity. We also used the statistic of I^2 to detect the degree of heterogeneity, with I2 $<$ 25%, 25%–75% and $>$75% to represent low, moderate and high degree of inconsistency, respectively[43,44]. In the analysis of pooled data, we used two different models according to the trait of the included studies: If no heterogeneity was found, a fixed effect model was adopted to determine the gene effect or the random effect model was used[45,46]. What's more, if heterogeneity across studies existed, subgroup analysis was performed to seek out the source of heterogeneity. Studies were subdivided by ethnicity (Caucasian versus Asian), genotyping methods (RFLP versus others), age (unmatched versus matched), smoking index (unmatched versus matched), lung function of COPD cases ($FEV_1 > 50\%$ prediction versus $FEV_1 < 50\%$ prediction) to find the source of any heterogeneity.

Hardy–Weinberg equilibrium (HWE) was tested in healthy control within each study. Deviation from HWE was tested using the χ^2 test. Studies with controls that depart from HWE ($p < 0.05$) were subjected to a sensitivity analysis in order to check the consistency of the overall effect.

We made use of Begg's funnel plot to examine the underlying publication bias, and also used Egger's weighted regression method to calculate P for bias[47,48]. If no publication bias existed, the funnel plot looked symmetrical.

All analyses were conducted with the use of Review Manager, V.5.0 (Revman, The Cochrane Collaboration) or STATA software, V.10.0 (STATA Corp).

1. Decramer, M., Janssens, W. & Miravitlles, M. Chronic obstructive pulmonary disease. *Lancet* **379**, 1341–1351 (2012).
2. Foreman, M. G., Campos, M. & Celedón, J. C. Genes and chronic obstructive pulmonary disease. *Med Clin North Am* **96**, 699–711 (2012).
3. Zitka, O. *et al.* Matrix metalloproteinases. *Curr Med Chem* **17**, 3751–3768 (2010).
4. Churg, A. *et al.* An MMP-9/-12 inhibitor prevents smoke-induced emphysema and small airway remodeling in guinea pigs. *Thorax* **62**, 706–713 (2007).
5. Churg, A. *et al.* Alpha1-antitrypsin suppresses TNF-a and MMP-12 production by cigarette smoke-stimulated macrophages. *Am J Respir Cell Mol Biol* **37**, 144–151 (2007).
6. Le Que'ment, C. *et al.* The selective MMP-12 inhibitor, AS111793 reduces airway inflammation in mice exposed to cigarette smoke. *Br J Pharmacol* **154**, 1206–1215 (2008).
7. Mercer, B. A., Wallace, A. C., Brinckerhoff, C. E. & D'Armiento, J. M. Identification of a cigarette smoke-responsive region in the distal MMP-1 promoter. *Am J Respir Cell Mol Biol* **40**, 4–12 (2009).
8. Atkinson, J. J. *et al.* The role of matrix metalloproteinase-9 in cigarette smoke-induced emphysema. *Am J Respir Crit Care Med* **183**, 876–884 (2011).
9. D'Armiento, J., Dalal, S. S., Okada, Y., Berg, R. A. & Chada, K. Collagenase expression in the lungs of transgenic mice causes pulmonary emphysema. *Cell* **71**, 955–961 (1992).
10. Hautamaki, R. D., Kobayashi, D. K., Senior, R. M. & Shapiro, S. D. Macrophage elastase is required for cigarette smoke-induced emphysema in mice. *Science* **277**, 2002–2004 (1997).
11. Nerusu, K. C. *et al.* Matrix metalloproteinase-3 (stromelysin-1) in acute inflammatory tissue injury. *Exp. Mol. Pathol* **83**, 169–176 (2007).
12. Cai, J., Wang, L. & Wang, A. MMP-1 gene polymorphsim and COPD susceptibility in Han nationality in Jiangxi province. *Shandong Medical Journal* **50**, 76–77 (2010).
13. Cheng, S. L., Yu, C. J. & Yang, P. C. Genetic polymorphisms of cytochrome p450 and matrix metalloproteinase in chronic obstructive pulmonary disease. *Biochem Genet* **47**, 591–601 (2009).
14. van Diemen, C. C. *et al.* Genetic variation in TIMP1 but not MMPs predict excess FEV1 decline in two general population-based cohorts. *Respir Res* **12**, 57 (2011).
15. Enewold, L., Mechanic, L. E., Bowman, E. D., Platz, E. A. & Alberg, A. J. Association of Matrix Metalloproteinase-1 Polymorphisms with Risk of COPD and Lung Cancer and Survival in Lung Cancer. *Anticancer Res* **32**, 3917–3922 (2012).
16. Han, W., Kuang, J. & Rao, W. MMP-9 polymorphisms and susceptibility to chronic obstructive pulmonary disease. *Chin J Tuberc Respir Dis* **29**, 277–278 (2006).
17. Haq, I. *et al.* Association of MMP-2 polymorphisms with severe and very severe COPD: a case control study of MMPs-1, 9 and 12 in a European population. *BMC Med Genet* **11**, 7–12 (2010).
18. Hersh, C. P. *et al.* Attempted replication of reported chronic obstructive pulmonary disease candidate gene associations. *Am J Respir Cell Mol Biol* **33**, 71–78 (2005).
19. Hua, D. *et al.* Association of MMP9 polymorphisms with susceptibility to chronic obstructive pulmonary disease in Ethnic Tibetan population. *Int J Respir* **30**, 1157–1160 (2010).
20. Ito, I. *et al.* Matrix metalloproteinase-9 promoter polymorphism associated with upper lung dominant emphysema. *Am J Respir Crit Care Med* **172**, 1378–1382 (2005).
21. Korytina, G. F., Akhmadishina, L. Z., Ianbaeva, D. G. & Viktorova, T. V. Polymorphism in Promoter Regions of Matrix Metalloproteinases (MMP1, MMP9, and MMP12) in Chronic Obstructive Pulmonary Disease Patients. *Russian Journal of Genetics* **44**, 202–208 (2008).
22. Korytina, G. F. *et al.* Association of MMP3, MMP9, ADAM33, and TIMP3 Polymorphisms with Chronic Obstructive Pulmonary Disease and Its Progression. *Molecular Biology* **46**, 438–449 (2012).
23. Lee, S. Y. *et al.* Polymorphisms in matrix metalloproteinase-1,-9 and -12 genes and the risk of chronic obstructive pulmonary disease in a Korean population. *Respiration* **80**, 133–138 (2010).
24. Minematsu, N., Nakamura, H., Tateno, H., Nakajima, T. & Yamaguchi, K. Genetic polymorphism in matrix metalloproteinase-9 and pulmonary emphysema. *Biochem Biophys Res Commun* **289**, 116–119 (2001).
25. Santus, P. *et al.* Stromelysin_1 polymor_ phism as a new potential risk factor in progression of chronic obstructive pulmonary disease. *Monaldi Arch Chest Dis* **71**, 15–20 (2009).
26. Schirmer, H., Basso da Silva, L., Moreira, J. S., Moreira, A. L. & Simon, D. Matrix metalloproteinase gene polymorphisms: lack of association with chronic obstructive pulmonary disease in a Brazilian population. *Genet Mol Res* **8**, 1028–1034 (2009).
27. Sun, P., Chen, J., Chen, R., Jin, J. & Li, Y. The relevance of matrixmetalloproteinase-1 gene polymorphism with chronic obstructive pulmonary disease susceptibility in Han nationality of northern China. *Chin J Respir Crit Care Med* **4**, 342–344 (2005).
28. Sun, C., Wang, W., Yang, J., Siqingaowa & Wang, L. The association of matrix metalloproteinase 3,12 polymorphism with susceptibility of chronic obstructive pulmonary disease. *Chin J CIinicians* (EIectronic Edition) **6**, 5826–5830 (2012).
29. Tesfaigzi, Y. *et al.* Genotypes in matrix metalloproteinase 9 are risk factors for COPD. *Int J Chron Obstruct Pulmon Dis* **1**, 267–278 (2006).
30. Zhang, R. *et al.* Matrix metalloproteinase polymorphisms and susceptibility to chronic obstructive pulmonary disease in Han nationality of north China. *Journal of Cardiovascular and Pulmonary Diseases* **23**, 240–243 (2004).
31. Zhang, R., He, Q., Yang, R., Lu, B. & Liu, Y. Study on matrix metalloproteinase 1, 9, 12 polymorphisms and susceptibility to chronic obstructive pulmonary disease among Han nationality in Northern China. *Chin J Epidemiol* **26**, 907–910 (2005).
32. Zhou, M. *et al.* Genetic polymorphism in matrix metalloproteinase-9 and the susceptibility to chronic obstructive pulmonary disease in Han population of south China. *Chin Med J (Engl)* **117**, 1481–1484 (2004).
33. Pillai, S. G. *et al.* A genome-wide association study in chronic obstructive pulmonary disease (COPD): identification of two major susceptibility loci. *PLoS Genet* **5**, e1000421 (2009).
34. Cho, M. H. *et al.* Variants in FAM13A are associated with chronic obstructive pulmonary disease. *Nat Genet* **42**, 200–202 (2010).
35. Kong, X. *et al.* Genome-wide association study identifies BICD1 as a susceptibility gene for emphysema. *Am J Respir Crit Care Med* **183**, 43–49 (2011).
36. Churg, A., Zhou, S. & Wright, J. L. Matrix metalloproteinases in COPD. *Eur Respir J* **39**, 197–209 (2012).
37. Demedts, I. K. *et al.* Elevated MMP-12 protein levels in induced sputum from patients with COPD. *Thorax* **61**, 196–201 (2006).
38. Vornooy, J. H. J., Lindeman, J. H. N., Jacobs, J. A., Hanemaaijer, R. & Wouters, E. F. Increased activity of matrix metalloproteinase -8 and matrix metalloproteinase -9 in induced sputum from patients with COPD. *Chest* **126**, 1802–1810 (2004).
39. Culpitt, S. V., Rogers, D. F., Traves, S. L., Barnes, P. J. & Donnelly, L. E. Sputum matrix metalloproteases: comparison between chronic obstructive pulmonary disease and asthma. *Respir Med* **99**, 703–710 (2005).

40. Louhelainen, N. *et al.* Elevation of sputum matrix metalloproteinase-9 persists up to 6 months after smoking cessation: a research study. *BMC Pulm Med* **10**, 13 (2010).
41. Joos, L. *et al.* The role of matrix metalloproteinase polymorphisms in the rate of decline in lung function. *Hum Mol Genet* **11**, 569–576 (2002).
42. Hunninghake, G. M. *et al.* MMP12, lung function, and COPD in high-risk populations. *N Engl J Med* **361**, 2599–2608 (2009).
43. Higgins, J. P. & Thompson, S. G. Quantifying heterogeneity in a meta analysis. *Stat Med* **21**, 1539–1558 (2002).
44. Higgins, J. P., Thompson, S. G., Deeks, J. J. & Altman, D. G. Measuring inconsistency in meta-analyses. *BMJ* **327**, 557–560 (2003).
45. Wang, J. H. *et al.* Polymorphisms of matrix metalloproteinases in myocardial infarction: a meta-analysis. *Heart* **97**, 1542–1546 (2011).
46. Yan, F. G. *et al.* Association between polymorphism of glutathione S-transferase P1 and chronic obstructive pulmonary disease: A meta-analysis. *Respiratory Medicine* **104**, 473–480 (2010).
47. Begg, C. B. & Mazumdar, M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* **50**, 1088–1101 (1994).
48. Egger, M., Davey, S. G. & Schneider, M. Bias in meta-analysis detected by a simple, graphical test. *BMJ* **315**, 629–634 (1997).

Acknowledgements

This work was supported in part by grants from the State Key Program of National Natural Science Foundation of China (No.81130001), National Basic Research Program of China (No.2009CB522103), National Key Technologies R&D Program for the 12th Five-year Plan (No.2012BAI05B01).

Author contributions

H.Z. have contributed to the design of the study, analysis and interpretation of data and drafting a part of manuscript. Y.W. also took part in collecting and analyzing data, and drafting a part of manuscript. Y.J., J.Z., C.Z. and L.C. searched the related papers and extracted data. J.J. carried out statistical analysis and revised manuscript. Z.C. prepared all figures. W.L. and H.S. designed this study and revised manuscript. All authors reviewed the manuscript.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

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

How to cite this article: Zhou, H. *et al.* Genetic Polymorphism of Matrix Metalloproteinase Family and Chronic Obstructive Pulmonary Disease Susceptibility: a Meta-analysis. *Sci. Rep.* **3**, 2818; DOI:10.1038/srep02818 (2013).