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Genetic variation in TIMP1 but not MMPs predict excess FEV\textsubscript{1} decline in two general population-based cohorts

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Abstract

**Background:** An imbalance in Matrix MetalloProteases (MMPs) and Tissue Inhibitors of MMPs (TIMPs) contributes to Chronic Obstructive Pulmonary Disease (COPD) development. Longitudinal studies investigating Single Nucleotide Polymorphisms (SNPs) in MMPs and TIMPs with respect to COPD development and lung function decline in the general population are lacking.

**Methods:** We genotyped SNPs in MMP1 (G-1607GG), MMP2 (-1306 C/T), MMP9 (3 tagging SNPs), MMP12 (A-82G and Asn357Ser) and TIMP1 (Phe124Phe and Ile158Ile) in 1390 Caucasians with multiple FEV\textsubscript{1} measurements from a prospective cohort study in the general population. FEV\textsubscript{1} decline was analyzed using linear mixed effect models adjusted for confounders. Analyses of the X-chromosomal TIMP1 gene were stratified according to sex. All significant associations were repeated in an independent general population cohort (n = 1152).

**Results:** MMP2 -1306 TT genotype carriers had excess FEV\textsubscript{1} decline (-4.0 ml/yr, p = 0.03) compared to wild type carriers. TIMP1 Ile158Ile predicted significant excess FEV\textsubscript{1} decline in both males and females. TIMP1 Phe124Phe predicted significant excess FEV\textsubscript{1} decline in males only, which was replicated (p = 0.10) in the second cohort. The MMP2 and TIMP1 Ile158Ile associations were not replicated. Although power was limited, we did not find associations with COPD development.

**Conclusions:** We for the first time show that TIMP1 Phe124Phe contributes to excess FEV\textsubscript{1} decline in two independent prospective cohorts, albeit not quite reaching conventional statistical significance in the replication cohort. SNPs in MMPs evidently do not contribute to FEV\textsubscript{1} decline in the general population.

Background

Chronic Obstructive Pulmonary Disease (COPD) is characterized by chronic airway inflammation, associated with extracellular matrix (ECM) degradation and loss of elastic recoil of lung tissue. The Matrix Metalloprotease (MMP) gene family is thought to participate in the excessive collagenolytic and elastolytic activity that contributes to ECM destruction. MMPs are a family of secreted and membrane associated zinc-dependent endopeptidases, capable of cleaving ECM and non-matrix proteins. Many studies have shown that MMP1, MMP2, MMP9, MMP12 protein and mRNA levels are higher in lung tissue and induced sputum of COPD patients than of controls [1-6].

Proteolytic activities of the MMPs are normally tightly controlled in several ways, e.g. by transcriptional regulation, activation of latent zymogen and interaction with endogenous inhibitors of MMPs, the Tissue Inhibitors of MMPs (TIMPs). Especially the imbalance between MMPs and TIMPs has been proposed to play a major role in ECM destruction and development of COPD, a pulmonary disease strongly associated with smoking. While most COPD patients have smoked, only a subset of smokers develops COPD, and it is likely that the susceptibility to smoking is genetically determined. It is thus reasonable that genetic determinants of the balance between MMPs and TIMPs contribute to COPD development.

Single nucleotide polymorphisms (SNPs) have been described in the promoter regions of MMP1, MMP2, MMP9 and MMP12 and they can alter their expression levels [7-10]. Joos et al. showed that SNPs in the MMP1
and MMP12 promoter regions are more prevalent in subjects with fast FEV\textsubscript{1} decline compared to subjects with no FEV\textsubscript{1} decline in a cohort of current smokers with mild to moderate airway obstruction [11]. SNPs in MMP12 have been variably associated with lung function, i.e. with higher lung function in children with asthma and adult smokers and additionally with a reduced risk of COPD in adult smokers [12] and increased risk of severe COPD [13]. A MMP9 promoter SNP has been associated with emphysema in a case-control study in a Japanese population [13] and with COPD in a Chinese population [14]. In contrast, the promoter SNP in MMP2, a biologically plausible candidate for COPD, as well as TIMP1 and TIMP2 SNPs have not been studied in relation to COPD development or FEV\textsubscript{1} decline. Whereas TIMP2 does not contain SNPs known to alter function or expression, two synonymous TIMP1 SNPs in the gene region responsible for binding and inactivating of MMP9 have been associated with asthma [15]. Thus, given the role of TIMP proteins in inhibiting effects of metalloproteases SNPs in these genes can conceivably play a role in COPD development.

Unraveling the genetics of MMPs and TIMPs in COPD development may identify subjects who may specifically benefit from novel treatments like synthetic MMP inhibitors that effectively prevent smoke-induced COPD in animal models. Therefore, we studied SNPs in MMP1, MMP2, MMP9, MMP12, and TIMP1 and their interaction in relation to accelerated FEV\textsubscript{1} decline and COPD development in a general population cohort. To verify our findings, we investigated whether significant associations could be replicated in an independent cohort of the general population.

### Methods

#### Subjects

We genotyped DNA from 1390 subjects of the Vlagtwedde/Vlaardingen cohort that participated in the last survey in 1989/1990 [16]. This general population-based cohort of Caucasians of Dutch descent started in 1965 and surveys were performed at three year intervals. At each survey, lung function measurements were performed using standardized protocols and questionnaires were completed (see additional file 1). The selection of the cohort and details of the study have been described previously [16]. The study protocol was approved by the local university hospital’s medical ethics committee and participants gave written informed consent.

As a replication cohort we used data from a random sample of 1152 subjects from the Doetinchem cohort, which is part of the larger MORGEN study [17,18]. The MORGEN study was a random sample of the general population of the Netherlands. Participants of the Doetinchem study underwent spirometry in 1994-1997 and 5 years later in 1999-2003. Characteristics of both study populations are presented in table 1. We identified subjects with COPD using the GOLD criteria (GOLD stage II or higher, i.e. FEV\textsubscript{1}/VC< 70% and FEV\textsubscript{1}<80% predicted) [19].

#### DNA collection and genotyping

DNA collection and the genotyping protocol of the Vlagtwedde/Vlaardingen study have been described previously [16]. We genotyped functional SNPs G-1607GG in MMP1, C-1306T in MMP2, A-82G and Asn357Ser (A/G) in MMP12. No tagging SNPs are known for TIMP1, therefore we decided to genotype two noncoding SNPs, previously associated with asthma [15]. Phe124Phe (T/C) and Ile158Ile (C/T) in TIMP1. In TIMP2, we genotyped G-418C. With Haplovew, using genotype data from the HapMap project [20,21] we selected 3 haplotype tagging SNPs for MMP9 that tag haplotypes with a frequency above 5% in MMP9 including 5 kb flanking regions at both the 3’UTR and 5’UTR: rs6065912, rs3918278 and rs8113877. Characteristics of the genotyped SNP are presented in table 2.

The SNPs that were significantly associated with excess FEV\textsubscript{1} decline or COPD development in the Vlagtwedde/Vlaardingen population were genotyped in the Doetinchem cohort by KBioscience http://www.kbioscience.co.uk using a patent-protected system (KASPar). We used the statistical software R, “genetics” package (version 1.9.1) to determine whether the SNPs were in Hardy Weinberg equilibrium and linkage disequilibrium.

#### Statistics

All TIMP1 analyses were stratified according to sex, since this gene is located on the X-chromosome. To investigate the effect of SNPs on annual FEV\textsubscript{1} decline in the Vlagtwedde/Vlaardingen cohort, we used Linear Mixed Effect (LME) models with adjustment for potential confounders (i.e. sex, first FEV\textsubscript{1} after age 30 years, pack-years) using a general genetic model (see additional file 1) [16,22]. We tested whether there was an interactive effect of TIMP1 and MMP SNPs on FEV\textsubscript{1} decline by introducing their interaction term into the model. We used ANOVA and linear regression models to study SNP effects on first and last available FEV\textsubscript{1} and FEV\textsubscript{1}/VC (adjusted for sex, age, pack-years, and height in regression models). Differences in genotype frequencies of single SNPs for all genes and additionally haplotype frequencies in MMP9 between subjects with and without COPD were tested using Chi-square tests.

The SNPs that were significantly associated with excess FEV\textsubscript{1} decline or COPD development in the Vlagtwedde/Vlaardingen population (p values < 0.05; tested 2-sided) were genotyped in the Doetinchem cohort for verification. FEV\textsubscript{1} decline in the Doetinchem cohort was calculated based on FEV\textsubscript{1} decline between the two surveys and genotype effects were tested using linear regression analyses, adjusted for sex, age, pack-years and baseline FEV\textsubscript{1}.  

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Statistical analyses were performed using SPSS (version 14.0.1 for Windows), S-Plus (version 7), the statistical package R (version 1.9.1) [23] and Chaplin [24,25].

**Results**

Allelic frequencies for the minor alleles of the MMP and TIMP SNPs in the Vlagtwedde/Vlaardingen cohort were comparable to those reported in the NCBI dbSNP database: MMP1 G-1607GG 0.51, MMP2 C-1306T 0.27, MMP9 rs3918278 0.03, MMP9 rs6065912 0.12, MMP9 rs8113877 0.40, MMP12 A-82G 0.15, MMP12 Asn357Ser 0.03, TIMP1 Ile158Ile in males 0.01, in females 0.01, and TIMP1 Phe124Phe in males 0.50, and in females 0.53. All SNPs were in Hardy Weinberg equilibrium.

The SNPs in MMP9 were in high LD (r$^2$ >0.8).

Association of MMP SNPs in Vlagtwedde/Vlaardingen

MMP2 C-1306T was significantly associated with accelerated longitudinal decline in FEV$_1$ in the total population (TT-genotype -4.0 ml/yr excess decline compared to CC-genotype, p = 0.027, figure 1), and was also associated with a lower mean FEV$_1$ % predicted (CC: 92.5, CT: 93.5, TT: 88.5% predicted; p = 0.013) at the last survey. This association remained significant after adjustment for packyears of smoking in linear regression models. SNPs in MMP1, MMP12 and SNPs and haplotypes in MMP9 were not significantly associated with longitudinal FEV$_1$ decline, level of lung function or presence of COPD (GOLD stage ≥ II) (table 3), although power was limited for the latter. Since smoking upregulates MMP activity [26], we also analyzed FEV$_1$ decline with respect to interaction of the SNPs and smoking. These interaction-terms were not significant.

Association of TIMP1 SNPs in Vlagtwedde/Vlaardingen

The TIMP1 Phe124Phe SNP was associated with excess FEV$_1$ decline in males only (-4.2 ml/yr excess FEV$_1$ decline compared to wild type (p = 0.041, figure 2). We

| Table 1 Characteristics of the Vlagtwedde/Vlaardingen and Doetinchem cohorts |
|---------------------------------|-----------------|-----------------|--------|
| | Vlagtwedde/Vlaardingen N = 1390 | Doetinchem N = 1152 |
| Age at last survey, yrs | 52 (35–79) | 50 (31–71) |
| Males, % | 51 | 47 |
| Pack-years | 9.0 (0–262.1) | 5 (0–84) |
| Never smokers, n (%) | 445 (32.0) | 371 (32.2) |
| Last FEV$_1$/Spred* | 93.5 (36.0–138.0) | 106.6 (39.1–150.7) |
| ΔFEV$_1$, ml/yr$^1$ | -21.1 (-121;155) | -27.8 (-292;130) |
| FEV$_1$, values, n | 7 (1–8) | 2 (2–2) |
| Yrs of follow-up | 21 (0–25) | 5 (5–5) |
| GOLD stage ≥ II,n (%) | 186 (13.4) | 37 (3.2) |

* FEV$_1$ % predicted at the last surveys of Vlagtwedde/Vlaardingen cohort (1989/1990) and the Doetinchem cohort (1999–2003).

$^1$ calculated as last-first FEV$_1$/years participated.

Data are presented as median (range).

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| Table 2 Characteristics of the genotyped SNPs |
|---------------------------------|-----------------|-----------------|--------|
| SNP name | rs number | Chromosome position of gene | Functionality |
| MMP1 G-1607GG | rs1799750 | 11q22-q23 | G-insertion generates a new 5’-GGA-3’ core recognition sequence for members of the ETS family of transcription factors |
| MMP2 C-1306T | rs243865 | 16q23 | T-allele disrupts a Sp1 binding site, thereby lowering the promoter activity approximately twofold in macrophages and epithelial cells |
| MMP9 rs6065912 | rs6065912 | 20q11.2-13.1 | tagging SNP for MMP9 gene |
| MMP9 rs3918278 | rs3918278 | | tagging SNP for MMP9 gene |
| TIMP1 Ile158Ile | rs11551797 | | unknown |
| TIMP2 G-418C | | | unknown |

Abbreviations: SNP Single Nucleotide Polymorphism; MMP Matrix Metallo Protease; TIMP Tissue Inhibitor of MMP; ETS E26 transformation-specific; Sp1 Stimulating Protein 1; AP-1 Activator Protein-1
found that the TIMP1 Ile158Ile SNP was associated with excess longitudinal FEV\textsubscript{1} decline in both males and females (-30.7 ml/yr respectively -9.5 ml/yr excess FEV\textsubscript{1} decline compared to wild type, p = 0.001 and p = 0.031 respectively, figure 2). The minor allele of the Ile158Ile SNP was more prevalent in females with COPD than without COPD: CT genotype, 6.5% and 1.5% respectively, p = 0.051 (table 4). The TIMP1 Phe124Phe SNP was not associated with COPD, although power to detect such an association was low. SNPs in TIMP1 were not associated with level of lung function cross-sectionally.

Interaction of TIMP1 and MMP genes on FEV\textsubscript{1} decline in Vlagtwedde/Vlaardingen
We found significant associations of TIMP1 and MMP2 SNPs with FEV\textsubscript{1} decline. To test for interaction between these genes, we included interaction terms of TIMP1 and MMP2 SNPs in our models on FEV\textsubscript{1} decline, and stratified the analyses by sex. These interaction terms were not significant.

Replication of significant findings in an independent population cohort
To investigate whether results were not found due to chance, we analyzed genes that were significantly associated with FEV\textsubscript{1} level or decline in the Vlagtwedde/Vlaardingen cohort, i.e. MMP2 and TIMP1, in an independent cohort of the general population. Genotype frequencies in the Doetinchem population were similar and not statistically different from the Vlagtwedde/Vlaardingen population (table 5). The TIMP1 Phe124Phe SNP was associated with excess FEV\textsubscript{1} decline in males (T allele -7.6 ml/yr compared to wild type, p = 0.10), similar to the findings in the Vlagtwedde/Vlaardingen cohort, although with lower significance. In contrast to the findings in Vlagtwedde/Vlaardingen, TIMP1 Ile158Ile was not associated with excess decline, but with less FEV\textsubscript{1} decline in females (42.9 ml/yr less decline compared to wild type, p = 0.008), but not in males. The MMP2 C-1306T was not significantly associated with excess FEV\textsubscript{1} decline or lower FEV\textsubscript{1} % predicted in the Doetinchem cohort.

To increase the power of the studies, we additionally tested for association for COPD development and FEV\textsubscript{1} % predicted in pooled analyses of the Vlagtwedde/Vlaardingen and Doetinchem cohorts. We only found a significant association for COPD with TIMP1 Ile158Ile in females (OR = 4.3, 95% CI = 1.2-15.3, p = 0.015), similar as the observation in Vlagtwedde/Vlaardingen alone but with stronger significance.
Discussion

Our study is the first to show that one SNP in TIMP1 predicts excess FEV\textsubscript{1} decline in two independent populations, albeit not quite reaching conventional statistical significance in the replication cohort. In the initial cohort we additionally found an association of MMP2 with FEV\textsubscript{1} decline, but this was not replicated in the second independent population, indicating that the role of genetic variation in MMP2 on rate of FEV\textsubscript{1} decline is still debatable. In contrast to previous reports on case-control studies that showed an association of MMP1, MMP9 and MMP12 with COPD, emphysema, decreased levels of FEV\textsubscript{1}, and/or excess decline in FEV\textsubscript{1} [12,14], we found no indication whatsoever for a role of MMP1, MMP9 or MMP12 in the development of (mild to moderate) COPD or FEV\textsubscript{1} decline in our prospective population studies. Consequently, our data suggest that the imbalance in MMPs and TIMPs is likely not disturbed due to genetic variations in MMP genes. This does not rule out that MMPs play a role in COPD development at all. Genetic variations in genes involved in regulation of MMPs and TIMPs levels, such as interleukin(IL)-10, IL-13, epithelial growth factor (EGF) and tumor necrosis factor-\alpha (TNF-\alpha) [27,28] may clearly influence the imbalance of MMPs and TIMPs in COPD. Future studies are needed that address the effect of these genes on FEV\textsubscript{1} decline in the general population.

We show for the first time that genetic variation in TIMP1 may accelerate the normally occurring FEV\textsubscript{1} decline in the general population. We found that the

| Table 3 | MMP SNPs and development of COPD in Vlagtwedde/Vlaardingen (GOLD stage ≥ II) |
|---------|---------------------------------|
| SNP     | No COPD N (%) | COPD N (%) | P value | SNP     | No COPD N (%) | COPD N (%) | P value |
| MMP1 G-1607GG | G | 295 (26.4) | 44 (24.7) | 0.845 | MMP9 rs6065912 | TT | 873 (77.0) | 145 (80.1) | 0.576 |
|         | GG | 565 (50.6) | 94 (52.8) | |         | TC | 245 (21.6) | 18 (33.2) | |
|         | GG | 257 (23.0) | 40 (22.5) | |         | CC | 16 (1.4) | 3 (1.7) | |
| MMP2 C-1306T | CC | 609 (52.8) | 103 (55.4) | 0.180 | MMP9 rs8113877 | TT | 408 (35.8) | 76 (40.6) | 0.137 |
|         | CT | 466 (40.5) | 65 (34.9) | |         | TC | 559 (49.0) | 77 (41.2) | |
|         | TT | 77 (6.7) | 18 (9.7) | |         | CC | 174 (15.2) | 34 (18.2) | |
| MMP12 Asn357Ser | AA | 1054 (93.1) | 171 (94.0) | 0.673 | MMP9 rs3918278 | GG | 1078 (94.7) | 177 (96.7) | 0.122 |
|         | AG | 78 (6.9) | 11 (6.0) | |         | GA | 59 (5.2) | 5 (2.7) | |
|         | GG | 0 (0) | 0 (0) | |         | AA | 1 (0.1) | 1 (0.6) | |
| MMP12 A-82G | AA | 812 (72.2) | 130 (71.4) | 0.831 | |
|         | AG | 281 (25.0) | 48 (26.4) | | |
|         | GG | 32 (2.8) | 4 (2.2) | | |

Figure 2 Effect of SNPs in TIMP1 on longitudinal decline in FEV\textsubscript{1}, stratified by sex. Mean adjusted declines in FEV\textsubscript{1} (in ml/yr) are shown per genotype; bars represent 95% confidence intervals.
common SNP Phe124Phe was associated with excess FEV₁ decline in males only. Of importance, this association was replicated in the Doetinchem cohort with a larger genotype effect (-9.0 ml/yr and -4.2 ml/yr excess FEV₁ decline in Doetinchem and Vlagtwedde/Vlaardingen respectively), but with somewhat lower significance (p values 0.10 and 0.04 respectively). Since the TIMP1 gene is located on the X-chromosome, carriage of one mutant allele may already account for an effect in males, whereas one mutant allele may be compensated by the other allele in females. However, another mechanism has to play a role since females homozygous for the mutant allele have a similar decline as heterozygous carriers.

Since the Phe124Phe SNP is a synonymous mutation and therefore unlikely having a functional effect on protein structure or function, it should be regarded as a marker for genetic variation in TIMP1. Future studies are warranted to identify SNPs that have a functional effect in this gene. Such SNPs may alter TIMP1 protein structure, resulting in an altered/diminished affinity for MMP9 and subsequently excess MMP9 activity leading to parenchymal destruction.

We observed opposite effects of the TIMP1 Ile158Ile SNP in the two populations under study. The SNP was associated with excess FEV₁ decline in both females and males in Vlagtwedde/Vlaardingen, and with less FEV₁ decline in females, without an effect in males in the Doetinchem cohort. The SNP has a very low prevalence and therefore type I errors can easily occur. By testing the SNP in an independent population, we can conclude that the significant effect in the Vlagtwedde/Vlaardingen population is possibly found by chance.

The MMP2 C-1306T genotype effect on FEV₁ decline is small in the Vlagtwedde/Vlaardingen cohort, but we observed no effect at all in the Doetinchem cohort, which may indicate that the association in Vlagtwedde/Vlaardingen may possibly be a spurious result that is not relevant on a population level. On the other hand, we can not completely rule out a genetic effect of MMP2 since the power to detect small genotype effects on longitudinal lung function decline is much larger in

### Table 4 TIMP1 SNPs and development of COPD in Vlagtwedde/Vlaardingen (GOLD stage ≥ II), stratified by sex

|       | FEMALES                          |        | FEMALES                          |        |        |        | FEMALES                          |        |       |        | FEMALES                          |        |       |
|-------|----------------------------------|--------|----------------------------------|--------|--------|--------|----------------------------------|--------|--------|--------|----------------------------------|--------|--------|
|       | No COPD N (%)                   | COPD N (%) | P value                          |        | No COPD N (%)                   | COPD N (%) | P value                          |        | No COPD N (%)                   | COPD N (%) | P value                          |
| TIMP1 Ile158Ile | CC 572 (98.5) | 43 (93.5) | 0.051                            |        | TIMP1 Ile158Ile | C 533 (98.9) | 130 (99.2) | 0.724                            |
|       | CT 9 (1.5)                      | 3 (6.5)  |                                  |        |       |        | T 6 (1.1)                        | 1 (0.8) |        |        | T 6 (1.1)                        | 1 (0.8) |        |
|       | TT 0 (0)                        | 0 (0)    |                                  |        |       |        |                                  |        |        |        |                                  |        |        |
| TIMP1 Phe124Phe | TT 122 (21.1) | 12 (25.0) | 0.298                            |        | TIMP1 Phe124Phe | T 264 (50.4) | 60 (45.8) | 0.348                            |
|       | TC 308 (53.2)                   | 20 (41.7) |                                  |        |       |        | C 260 (49.6) | 71 (54.2) |        |        |                                  |        |        |
|       | CC 149 (25.7)                   | 16 (33.3) |                                  |        |       |        |                                  |        |        |        |                                  |        |        |

### Table 5 Genotype distribution of MMP2 and TIMP1 SNPs in Vlagtwedde/Vlaardingen and Doetinchem

| Vlagtwedde/Vlaardingen | Doetinchem | P value |
|-------------------------|------------|---------|
| MMP2 C-1306T            |            |         |
| CC 734 (53.0)           | 609 (55.1) | 0.055   |
| CT 552 (39.9)           | 443 (40.1) |         |
| TT 98 (7.1)             | 53 (4.8)   |         |

|       | Vlagtwedde/Vlaardingen | Doetinchem | P value | Vlagtwedde/Vlaardingen | Doetinchem | P value |
|-------|------------------------|------------|---------|------------------------|------------|---------|
| FEMALES | TIMP1 Ile158Ile | CC 636 (98.0) | 602 (99.2) | 0.079 | TIMP1 Ile158Ile | C 686 (99.0) | 526 (99.4) | 0.394 |
|       | CT 13 (2.0)          | 5 (0.8)    |        | T 7 (1.0)              | 3 (0.6)    |        |
|       | TT 0 (0)             | 0 (0)      |        |                        |            |        |
| MALES | TIMP1 Phe124Phe | TT 138 (21.3) | 135 (23.4) | 0.657 | TIMP1 Phe124Phe | T 336 (49.6) | 240 (47.6) | 0.494 |
|       | TC 338 (52.0)        | 295 (51.1) |        | C 341 (50.4)           | 264 (52.4) |        |
|       | CC 173 (26.7)        | 147 (25.5) |        |                        |            |        |

TIMP1 SNP distributions are stratified by sex.
the Vlagtwedde/Vlaardingen population due to the substantial longer follow-up time than in Doetinchem [29]. This may explain the lack of replication. Further studies with comparable power as in Vlagtwedde/Vlaardingen are warranted to elucidate the role of MMP2 in FEV\textsubscript{1} decline in the general population.

MMP9 SNPs were not associated with development of COPD or FEV\textsubscript{1} decline in our study. We did not genotype the MMP9 C-1562T SNP that was previously associated cross-sectionally with the presence of emphysema or COPD in Japanese and Chinese individuals in a case-control study [13,14] due to technical problems. Therefore, we cannot rule out a genetic role of MMP9 in COPD development. However, we tagged the whole MMP9 gene for haplotypes with a frequency above 10\%, and found strong LD in the whole region. We are therefore confident that we also tagged the C-1562T SNP and that we did not miss information. Alternatively, the causative factor for higher levels of MMP9 in COPD lung tissue can be due to their transcriptional upregulation by other cytokines involved in COPD [30,31]. It is therefore of interest to analyze SNPs in these genes in the future as well.

We did not confirm associations of the MMP1 and MMP12 SNPs and lung function decline as previously described by Joos et al and Hunninghake et al [11,12]. However, in the first study the MMP12 Asn357Val SNP was only associated with rate of decline in FEV\textsubscript{1} in combination with the MMP1 G-1607GG SNP. We performed the same type of analyses and found no association. Since Hunninghake et al found associations of MMPs and lung function in smokers, we also performed such stratified analyses according to smoking, but found no associations in the ever or current smokers.

Although the role of MMPs in COPD pathogenesis has clearly been demonstrated, we are the first to analyze the effects of SNPs in a cohort of the general population, whereas previous studies have used case-control designs. Moreover, differences in phenotypes make the comparison of our study and previous studies difficult. For example, several studies have investigated the effect of the C-1562T SNP in MMP9 in smokers and nonsmokers with respect to emphysematous phenotypes using chest CT scans [13,14,30]. We do not have CT scans available in Vlagtwedde/Vlaardingen or in Doetinchem, so we can not assess such genetic effects since pulmonary function tests are not very sensitive to detect (mild) emphysema [31].

Since the selection of our SNPs was hypothesis-driven, and we tested only 9 SNPs, we feel that a correction for multiple testing is not warranted, moreover since the strength of the current study lies in the replication of significant findings of one cohort in a second cohort. We feel we did not miss any clinically relevant associations of greater than 5 ml/year excess FEV\textsubscript{1} decline due to lack of power. For example: we had approximately 1000 subjects with the wild type genotype of the rs8113877 in MMP9 and on average a mean annual decline in FEV\textsubscript{1} of 17 ml/yr which results in a 80\% power to detect an excess decline of 5.5 ml/yr in FEV\textsubscript{1} in mutant carriers (n = 300), assuming a SD of 30 (derived from the actual SE = 1.166) in both groups. However, we may have missed associations of MMP SNPs with COPD, since we only had 40\% power to detect an OR of 1.5, assuming a risk allele frequency of 0.25.

Conclusions

Our study shows that genetic variation in TIMP1 is associated with excess FEV\textsubscript{1} decline in two independent general populations, reaching moderate significance. Further research is needed to assess the functionality of this finding. We could not confirm a role for MMP SNPs in excess FEV\textsubscript{1} decline and COPD development in the general population, although our study had sufficient power to detect genetic effects. Since SNPs in MMP do not appear to contribute to COPD, it is of interest to assess the genetic contribution of MMP modifying genes, like IL-10, IL-13, EGF, and TNF-α that regulate transcription of MMPs. In addition, SNPs in other TIMPs, such as TIMP2, may also affect the MMP-TIMP balance and thereby exert an effect on FEV\textsubscript{1} decline in the general population.

Additional material

Addtional file 1: This manuscript contains an online supplement with additional methods.

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Authors’ contributions

CCD performed the lab work, statistical analyses and drafted the manuscript. DSP is co principal investigator of the project, obtained funding of and
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