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## MMP12, Lung Function, and COPD in High-Risk Populations

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### ABSTRACT

#### BACKGROUND

Genetic variants influencing lung function in children and adults may ultimately lead to the development of chronic obstructive pulmonary disease (COPD), particularly in high-risk groups.

#### METHODS

We tested for an association between single-nucleotide polymorphisms (SNPs) in the gene encoding matrix metalloproteinase 12 (MMP12) and a measure of lung function (prebronchodilator forced expiratory volume in 1 second [FEV<sub>1</sub>]) in more than 8300 subjects in seven cohorts that included children and adults. Within the Normative Aging Study (NAS), a cohort of initially healthy adult men, we tested for an association between SNPs that were associated with FEV<sub>1</sub> and the time to the onset of COPD. We then examined the relationship between MMP12 SNPs and COPD in two cohorts of adults with COPD or at risk for COPD.

#### RESULTS

The minor allele (G) of a functional variant in the promoter region of MMP12 (rs2276109 [−82A→G]) was positively associated with FEV<sub>1</sub> in a combined analysis of children with asthma and adult former and current smokers in all cohorts ( $P=2\times 10^{-6}$ ). This allele was also associated with a reduced risk of the onset of COPD in the NAS cohort (hazard ratio, 0.65; 95% confidence interval [CI], 0.46 to 0.92;  $P=0.02$ ) and with a reduced risk of COPD in a cohort of smokers (odds ratio, 0.63; 95% CI, 0.45 to 0.88;  $P=0.005$ ) and among participants in a family-based study of early-onset COPD ( $P=0.006$ ).

#### CONCLUSIONS

The minor allele of a SNP in MMP12 (rs2276109) is associated with a positive effect on lung function in children with asthma and in adults who smoke. This allele is also associated with a reduced risk of COPD in adult smokers.

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**M**MP-12 (MATRIX METALLOPROTEINASE 12, also known as macrophage metalloelastase or matrix metalloproteinase 12 [Mmp12] in mice) is produced by macrophages, the predominant cell type that patrols the lower airspaces under normal conditions and the main inflammatory cell type that is recruited with smoking.<sup>1</sup> Mmp12 is essential for the development of emphysema in mice exposed to cigarette smoke,<sup>2</sup> including mice with increased expression of interleukin-13.<sup>3</sup>

Increased expression of MMP12 in the lower airways can lead to degradation of elastin, resulting in elastin fragments that can cause a positive feedback loop, further increasing macrophage recruitment in mice<sup>4</sup> and in cultured human cells.<sup>5</sup> In humans, an increase in MMP12 expression by a factor of nearly 9 has been noted in alveolar macrophages from smokers as compared with those from nonsmokers.<sup>6</sup> Despite these findings, the role of MMP-12 in the development of emphysema in humans remains unclear.

Host characteristics and environmental exposures during childhood, including increased airway responsiveness, asthma, or both,<sup>7,8</sup> can influence lung function during adolescence and adulthood.<sup>7,8</sup> Because of the strong correlation among repeated measures of pulmonary function throughout life,<sup>7</sup> factors determining lung function during childhood may influence the risk of chronic obstructive pulmonary disease (COPD) in adults.<sup>9</sup>

We hypothesized that variants in MMP12 influence lung function and are risk factors for COPD, particularly in groups that are at risk for reduced lung function. To examine this hypothesis, we tested for an association between single-nucleotide polymorphisms (SNPs) in MMP12 and a measure of lung function (forced expiratory volume in 1 second [FEV<sub>1</sub>]) in seven cohorts, including participants in two family-based studies of children with asthma, participants in a birth-cohort study, and four cohorts that included adults with COPD and adults without COPD.

## METHODS

### STUDY DESIGN

A detailed description of the study methods can be found in the Supplementary Appendix, available with the full text of this article at NEJM.org.

The protocols for the recruitment of subjects and the collection of data in the Genetics of Asthma in Costa Rica Study (GACRS), the Childhood Asthma Management Program (CAMP, ClinicalTrials.gov number, NCT00000575), the Children, Allergy, Milieu, Stockholm, Epidemiological Survey (BAMSE), the Boston Early-Onset COPD (eoCOPD) study, the National Emphysema Treatment Trial (NETT, NCT00000606), the Lovelace Smokers Cohort (Lovelace), and the Normative Aging Study (NAS) have been described previously.<sup>10-16</sup> Spirometry was performed in accordance with American Thoracic Society recommendations.<sup>17</sup> All the studies were approved by an institutional review board or ethics committee. All adult participants and parents of children who participated gave written informed consent, and minors who were able to do so gave assent.

### GENOTYPING

In the GACRS, genotyped markers were selected with the use of a linkage-disequilibrium–tagging algorithm for MMP12 and its 10-kb flanks in Centre d'Etude du Polymorphisme Humain (CEPH) trios in HapMap. The same SNPs that were genotyped in the GACRS cohort were genotyped in the CAMP cohort to compare linkage-disequilibrium patterns between these cohorts. The two SNPs that showed significant associations in the GACRS cohort (rs737693 and rs2276109) were genotyped in the eoCOPD, NETT, and NAS cohorts; one of these SNPs (rs2276109) was genotyped in the BAMSE and Lovelace cohorts.

To determine whether our findings could be explained by linkage disequilibrium between variants in MMP12 and those in an adjacent gene encoding matrix metalloproteinase 3 (MMP3), we genotyped four SNPs in MMP3 in the GACRS cohort.

### STATISTICAL ANALYSIS

In all the cohorts, prebronchodilator FEV<sub>1</sub> (hereafter called FEV<sub>1</sub>) was analyzed as a continuous variable. We defined COPD according to modified stage II criteria of the Global Initiative for Chronic Obstructive Lung Disease.<sup>9</sup> We examined the time to the onset of COPD in the NAS cohort and COPD itself in the eoCOPD and Lovelace cohorts. All analyses were adjusted for age, sex, height, and exposure to smoke.

We performed family-based association analy-

ses of FEV<sub>1</sub>, COPD, or both in the GACRS and eoCOPD cohorts with the use of the family-based association test (FBAT) statistic implemented in PBAT software, version 3.6,<sup>18</sup> and linear regression analyses of FEV<sub>1</sub> in the BAMSE, NETT, and Lovelace cohorts with the use of SAS software, version 9.1.2 (SAS Institute). The family-based association analysis of repeated measures of FEV<sub>1</sub> in the CAMP cohort was performed with the use of the FBAT–Principal Components (PC) approach.<sup>19</sup> In the NAS cohort, we performed longitudinal analyses of FEV<sub>1</sub> using generalized linear mixed models and a survival analysis of COPD onset using Cox proportional-hazards models. We used logistic regression for the association analysis of COPD in the Lovelace cohort.

Given the results from the GACRS cohort, we performed all subsequent analyses under a dominant genetic model. P values for all tests in the replication cohorts were one-sided, since all associations in these cohorts were in the same direction as those in the GACRS cohort. Combined P values for all cohorts were calculated with the use of both Fisher's method and a weighted z-score method to account for differences in sample size among the study cohorts.<sup>20</sup> We estimated the population risk of COPD attributable to MMP12 genotypes and the discriminatory power of these genotypes to predict COPD onset in the NAS cohort.<sup>21</sup>

## RESULTS

### COHORTS

Baseline characteristics of the children in the GACRS, CAMP, and BAMSE cohorts and of the adults in the eoCOPD, NETT, Lovelace, and NAS cohorts are shown in Table 1. The characteristics of children in the GACRS and CAMP cohorts were similar to those of children in the BAMSE cohort, except that the proportions of children exposed to environmental tobacco smoke and of children with asthma were lower in the BAMSE cohort than in the CAMP or GACRS cohort. Whereas most of the adults in the eoCOPD and Lovelace cohorts were women, the majority of the adults in the NETT cohort and all the adults in the NAS cohort were men. The prevalence of current smoking was lower in the eoCOPD and NETT cohorts than in the NAS and Lovelace cohorts. The minor-allele frequency for SNP rs2276109 was similar in all cohorts. The

linkage-disequilibrium pattern for SNPs across MMP12 was similar in the GACRS and CAMP cohorts, and pairwise SNP comparisons were similar to those in CEPH trios from HapMap (Fig. 1 and Table 1 in the Supplementary Appendix).

### LUNG FUNCTION IN CHILD COHORTS

#### GACRS

The minor alleles of four contiguous SNPs in the 3' genomic region of MMP12 (rs586701, rs737693, rs654600, and rs674546) were significantly associated with increased FEV<sub>1</sub> (P values ranging from 0.02 to 0.001) (Fig. 1). Because one of these SNPs (rs737693) is in tight linkage disequilibrium ( $r^2=0.91$ ) with a functional polymorphism of MMP12 (rs2276109)<sup>24</sup> in CEPH trios in the HapMap (Fig. 1), we genotyped the latter SNP. As expected, the minor allele of rs2276109 was also positively associated with FEV<sub>1</sub> (Table 2). The minor allele of rs2276109 was associated with an increase in FEV<sub>1</sub> of 125 ml (95% confidence interval [CI], 46 to 203) among children with asthma (P=0.002). There was no association between SNPs in MMP3 and FEV<sub>1</sub>.

#### CAMP

Because CAMP was a clinical trial, we first tested for an association in all children and then in children stratified according to treatment group (budesonide vs. placebo or nedocromil). In the analysis that included all children, we observed no replication of the association observed in GACRS. We did, however, find significant associations between two of the SNPs associated with FEV<sub>1</sub> in the GACRS cohort (rs737693 and rs2276109) and FEV<sub>1</sub> in children who were taking budesonide (P=0.01 for both SNPs) (Table 2 and Fig. 1).

#### BAMSE

To determine whether our findings for lung function were limited to children with asthma, we used data from BAMSE, a birth cohort that was unselected for parental history of asthma or other diseases,<sup>13</sup> to test for an association between FEV<sub>1</sub> and one of the two SNPs that was significantly associated with FEV<sub>1</sub> in the GACRS and CAMP cohorts (rs2276109). We observed no significant association between SNP rs2276109 and FEV<sub>1</sub> measured at 8 years of age in the analysis of the total cohort. After stratification according to asthma status, we observed an association of

**Table 1. Baseline Characteristics of the Subjects in the Study Cohorts.\***

| Characteristic                                     | Child Cohorts    |                 |                   |                   | Adult Cohorts   |                      |                 |
|--|------------------|-----------------|-------------------|-------------------|-----------------|----------------------|-----------------|
|  | GACRS<br>(N=417) | CAMP<br>(N=503) | BAMSE<br>(N=1427) | eoCOPD<br>(N=127) | NETT<br>(N=378) | Lovelace<br>(N=1487) | NAS<br>(N=1468) |
| Age — yr   |                  |                 |                   |                   |                 |                      |                 |
| Median   | 8.7              | 8.6             | 8.4               | 49.4              | 68.0            | 56.1                 | 41.5            |
| Interquartile range                                | 7.7–10.4         | 7.0–10.5        | 8.0–8.7           | 46.1–55.6         | 64.0–71.0       | 48.8–64.0            | 35.7–46.9       |
| Female sex — no. (%)                               | 157 (37.6)       | 192 (38.2)      | 706 (49.5)        | 95 (74.8)         | 136 (36.0)      | 1223 (82.2)          | 0               |
| Asthma in children or COPD in adults — no. (%)     | 417 (100)        | 503 (100)       | 109 (7.6)         | 127 (100)         | 378 (100)       | 311 (20.9)           | 207 (14.1)†     |
| FEV <sub>1</sub> ‡                                 |                  |                 |                   |                   |                 |                      |                 |
| Volume — liters                                    |                  |                 |                   |                   |                 |                      |                 |
| Median   | 1.7              | 1.7             | 1.8               | 0.5               | 0.7             | 2.4                  | 3.9             |
| Interquartile range                                | 1.4–2.0          | 1.3–1.9         | 1.6–2.0           | 0.4–0.7           | 0.5–0.9         | 1.9–2.8              | 3.5–4.3         |
| Percent of predicted value                         |                  |                 |                   |                   |                 |                      |                 |
| Median   | —                | 95.0            | 105.6             | 18.3              | 25.0            | 90.0                 | 96.9            |
| Interquartile range                                | —                | 86.0–104.0      | 97.6–112.6        | 13.3–24.1         | 20.0–29.0       | 76.0–101.0           | 88.7–104.4      |
| Exposure to environmental tobacco smoke — no. (%)§ | 108 (25.9)       | 172 (34.2)      | 252 (17.7)        | 24 (18.9)         | —               | —                    | —               |
| Current smokers                                    |                  |                 |                   |                   |                 |                      |                 |
| Subjects — no. (%)                                 | 0                | 4 (0.8)¶        | 0                 | 16 (12.6)         | 0               | 823 (55.3)           | 506 (34.5)      |
| Pack-years — no.                                   |                  |                 |                   |                   |                 |                      |                 |
| Median   | —                | —               | —                 | 36                | 61.5            | 35.5                 | 10.5            |
| Interquartile range                                | —                | —               | —                 | 25–51             | 45–82           | 26–50                | 0–24            |
| Study visits — no.                                 |                  |                 |                   |                   |                 |                      |                 |
| Median   | Cross-sectional  | 11              | Cross-sectional   | Cross-sectional   | Cross-sectional | Cross-sectional      | 7               |
| Interquartile range                                |                  |                 |                   |                   |                 |                      | 6–9             |
| Follow-up period — yr                              |                  |                 |                   |                   |                 |                      |                 |
| Median   | Cross-sectional  | 4               | Cross-sectional   | Cross-sectional   | Cross-sectional | Cross-sectional      | 31.8            |
| Interquartile range                                |                  |                 |                   |                   |                 |                      | 25.4–34.6       |
| Minor-allele frequency                             |                  |                 |                   |                   |                 |                      |                 |
| rs737693   | 0.07             | 0.11            | Not determined    | 0.09              | 0.11            | Not determined       | 0.11            |
| rs2276109  | 0.07             | 0.09            | 0.13              | 0.10              | 0.12            | 0.13                 | 0.12            |

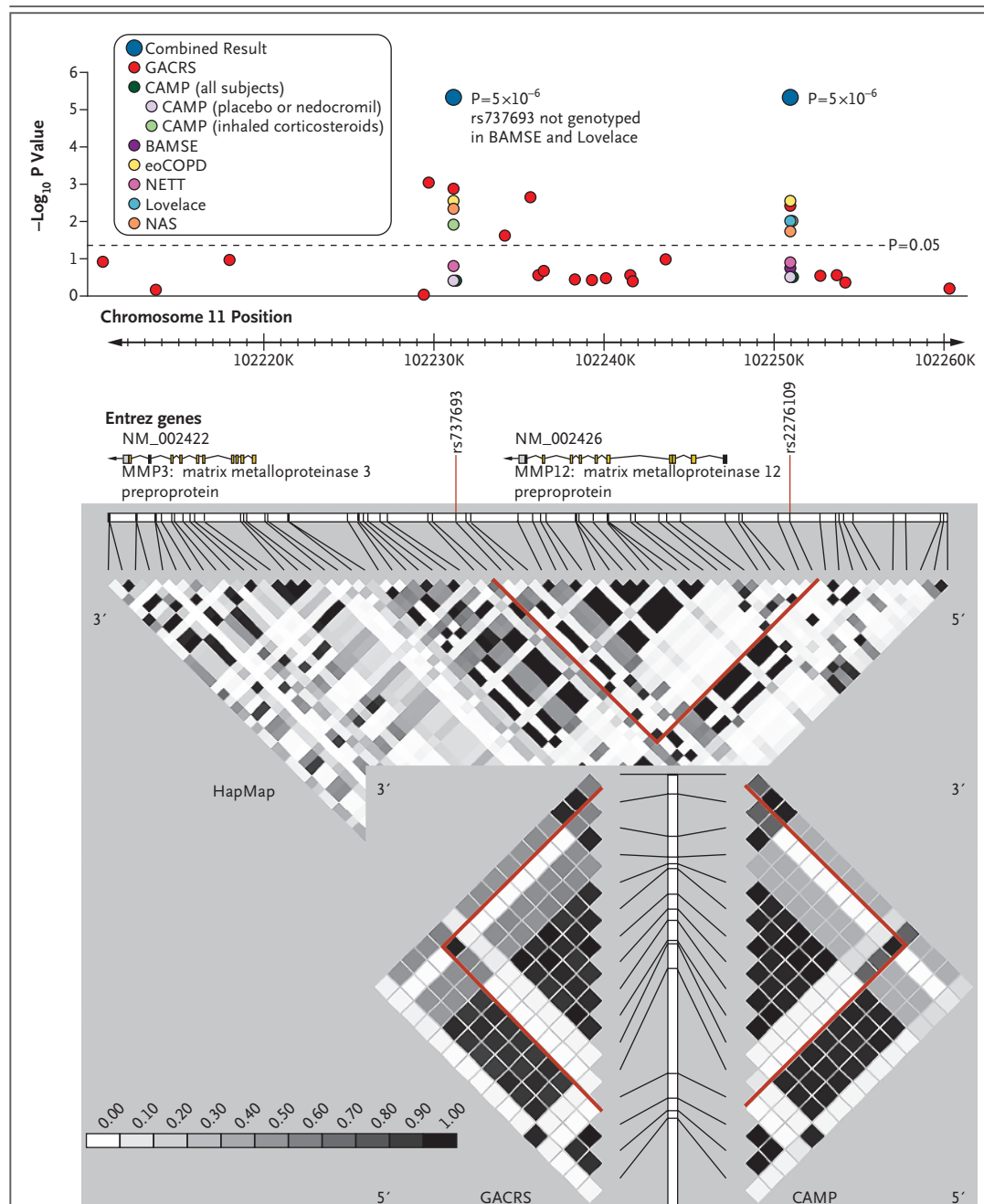
\* The child cohorts included index children (probands) in nuclear families in the Genetics of Asthma in Costa Rica Study (GACRS), index children in nuclear families in the Childhood Asthma Management Program (CAMP), and children in the Children, Allergy, Milieu, Stockholm, Epidemiological Survey birth cohort study (BAMSE). The adult cohorts included probands in families of subjects with severe chronic obstructive pulmonary disease (COPD) in the Boston Early-Onset COPD Study (eoCOPD); subjects in the genetics ancillary study of the National Emphysema Treatment Trial (NETT); subjects in the Lovelace Smokers Cohort (Lovelace); and subjects in the longitudinal Normative Aging Study (NAS). FEV<sub>1</sub> denotes forced expiratory volume in 1 second.

† The number includes all subjects in whom Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage II COPD developed (ratio of FEV<sub>1</sub> to forced vital capacity [FVC] of less than 0.7 and an FEV<sub>1</sub> of less than 80% of the predicted value, on the basis of prebronchodilator spirometry) during the NAS follow-up period of approximately 30 years.

‡ These are prebronchodilator values. The predicted values for FEV<sub>1</sub> are derived from Hankinson et al.<sup>22</sup> for white children in CAMP and BAMSE and from Crapo et al.<sup>23</sup> for adults in eoCOPD, NETT, Lovelace, and NAS. There are no predicted values for FEV<sub>1</sub> in Costa Ricans.

§ Exposure to environmental tobacco smoke was determined to be present if at least one person living in the child's household was a current smoker. This variable was not measured in the NETT, Lovelace, and NAS cohorts.

¶ All children who reported a positive smoking history at any visit in the CAMP trial are included here.



**Figure 1. Results of Analyses of the Association between SNPs in or near *MMP12* and  $FEV_1$ .**

The upper portion of the figure shows the results of the analyses of an association between prebronchodilator forced expiratory volume in 1 second ( $FEV_1$ ) and all single-nucleotide polymorphisms (SNPs) genotyped in subjects in the Genetics of Asthma in Costa Rica Study (GACRS) and between prebronchodilator  $FEV_1$  and relevant SNPs in subjects in the Childhood Asthma Management Program (CAMP); the Children, Allergy, Milieu, Stockholm Epidemiological Survey (BAMSE); the Boston Early-Onset COPD (eoCOPD) study; the National Emphysema Treatment Trial (NETT); the Lovelace Smokers Cohort (Lovelace); and the Normative Aging Study (NAS). P values for combined analyses were calculated with the use of Fisher's method. The bottom portion of the figure shows the pairwise ( $r^2$ ) linkage-disequilibrium patterns for the genotyped region in the Centre d'Etude du Polymorphisme Humain (CEPH) population and in the GACRS and CAMP parents. The red lines show the similarity in the linkage-disequilibrium patterns for rs737693 and rs2276109 in parents in CEPH trios in HapMap, GACRS, and CAMP.



**Table 2. Results of Analyses of an Association between the A→G Minor Allele of SNP rs2276109 in the Promoter Region of *MMP12* and FEV<sub>1</sub> in All Study Cohorts.\***

| Subjects   | Association between rs2276109 and FEV <sub>1</sub> , Expressed as P Values |                   |       |        |      |          |      |  |  |
|--|--|-------------------|-------|--------|------|----------|------|--|--|
|  | GACRS  | CAMP              | BAMSE | eoCOPD | NETT | Lovelace | NAS  | Combined Overall†                        | Combined Replication‡                    |
| All  | 0.004  | 0.40, 0.42, 0.01§ | 0.18  | 0.003  | 0.13 | 0.01     | 0.02 | 5×10 <sup>-6</sup> (2×10 <sup>-6</sup> ) | 2×10 <sup>-4</sup> (3×10 <sup>-5</sup> ) |
| Children with asthma and smokers with COPD or at risk for COPD | 0.004  | 0.40              | 0.07  | 0.005  | 0.13 | 0.01     | 0.01 | 2×10 <sup>-6</sup> (5×10 <sup>-7</sup> ) | 4×10 <sup>-5</sup> (1×10 <sup>-5</sup> ) |
| Children without asthma and adults who had never smoked        |  |                   | 0.28  | 0.28   |      |          | 0.43 | 0.34 (0.15)                              |  |

\* Included are nuclear families of children with asthma in the Genetics of Asthma in Costa Rica Study (GACRS); nuclear families of children with asthma in the Childhood Asthma Management Program (CAMP); children in the Children, Allergy, Milieu, Stockholm, Epidemiological Survey birth cohort study (BAMSE); families of subjects with severe chronic obstructive pulmonary disease (COPD) in the Boston Early-Onset COPD Study (eoCOPD); subjects in the genetics ancillary study of the National Emphysema Treatment Trial (NETT); subjects in the Lovelace Smokers Cohort (Lovelace); and subjects in the longitudinal Normative Aging Study (NAS). In each cohort, the association between the minor allele and prebronchodilator forced expiratory volume in 1 second (FEV<sub>1</sub>) was in a positive direction. SNP denotes single-nucleotide polymorphism.

† Combined overall P values were calculated with the use of Fisher's method. P values from a weighted z-score method are in parentheses.

‡ Combined replication P values were calculated with the use of Fisher's method after exclusion of data from the GACRS. P values from a weighted z-score method are in parentheses.

§ The second P value is for the analysis of children who were randomly assigned to receive either inhaled placebo or inhaled nedocromil. The third P value is for the analysis of children who were randomly assigned to receive an inhaled corticosteroid (budesonide).

borderline significance between the minor allele of rs2276109 and increased FEV<sub>1</sub> in children with asthma but no significant association in children without asthma (Table 2).

#### LUNG FUNCTION IN ADULT COHORTS

Reflecting our findings in children with asthma, the minor allele of SNP rs2276109 was significantly associated with increased FEV<sub>1</sub> in the adult cohorts of the eoCOPD study, Lovelace, and NAS (Table 2 and Fig. 1). Although we observed no significant association between this SNP and FEV<sub>1</sub> in the NETT cohort, the direction of the association between each SNP (rs2276109 and rs737693) and FEV<sub>1</sub> was the same as it was in the other cohorts (Table 2). We obtained similar results for the minor allele of SNP rs737693 in the eoCOPD and NAS cohorts (Fig. 1, and Table 2 in the Supplementary Appendix).

Among the adult cohorts, only the eoCOPD study (319 subjects) and NAS (477) included persons who had never smoked. To determine whether the association between SNP rs2276109 and FEV<sub>1</sub> is specific to smokers, we repeated the analyses in the eoCOPD and NAS cohorts after stratification according to smoking status. In these analyses, the minor allele of rs2276109

was significantly associated with increased FEV<sub>1</sub> in former smokers and current smokers but not in subjects who had never smoked (Table 2). Similar results were obtained for SNP rs737693 (Table 2 in the Supplementary Appendix).

#### LUNG FUNCTION IN ALL COHORTS

We observed a significant positive association between the minor allele of SNP rs2276109 and FEV<sub>1</sub> in a combined analysis of all cohorts, an association that remained significant in the analysis of the combined replication cohorts (i.e., all cohorts with the exception of the GACRS cohort) (Table 2 and Fig. 1). In the NAS cohort, the minor allele of rs2276109 was significantly associated with increased baseline FEV<sub>1</sub> but not with the rate of decline in lung function.

Because our findings in the analyses of subgroups in the BAMSE, eoCOPD, and NAS cohorts were significant only in children with asthma and adult smokers, we performed an analysis of all cohorts after excluding children without asthma (in BAMSE) and persons who had never smoked (in the eoCOPD study and NAS). In this analysis, there was enhanced evidence of a positive association between the minor allele of rs2276109 and FEV<sub>1</sub> (Table 2).

We obtained similar results for rs737693 and FEV<sub>1</sub> in a combined analysis of the five cohorts that had data for this SNP (Fig. 1, and Table 2 in the Supplementary Appendix).

#### COPD IN ADULT COHORTS

##### NAS

COPD developed in 207 of the 1468 subjects in the NAS cohort. We observed that the minor allele of rs2276109 was associated with a 35% reduction in the risk of the onset of COPD (hazard ratio, 0.65; 95% confidence interval [CI], 0.46 to 0.92;  $P=0.02$ ). Reciprocally, the absence of this allele (homozygosity for the major allele) was associated with a 54% increase in the risk of the onset of COPD. Survival curves according to MMP12 genotype (rs2276109) in all subjects and in subjects stratified according to smoking status at study entry are shown in Figure 2. Current smoking was associated with a 91% increase in the risk of the onset of COPD (hazard ratio, 1.91; 95% CI, 1.38 to 2.64;  $P<0.0001$  after adjustment for number of pack-years) and with an accelerated decline in lung function ( $P=0.001$  for the interaction between current smoking and time), findings that are consistent with the known role of smoking in the pathogenesis of COPD.

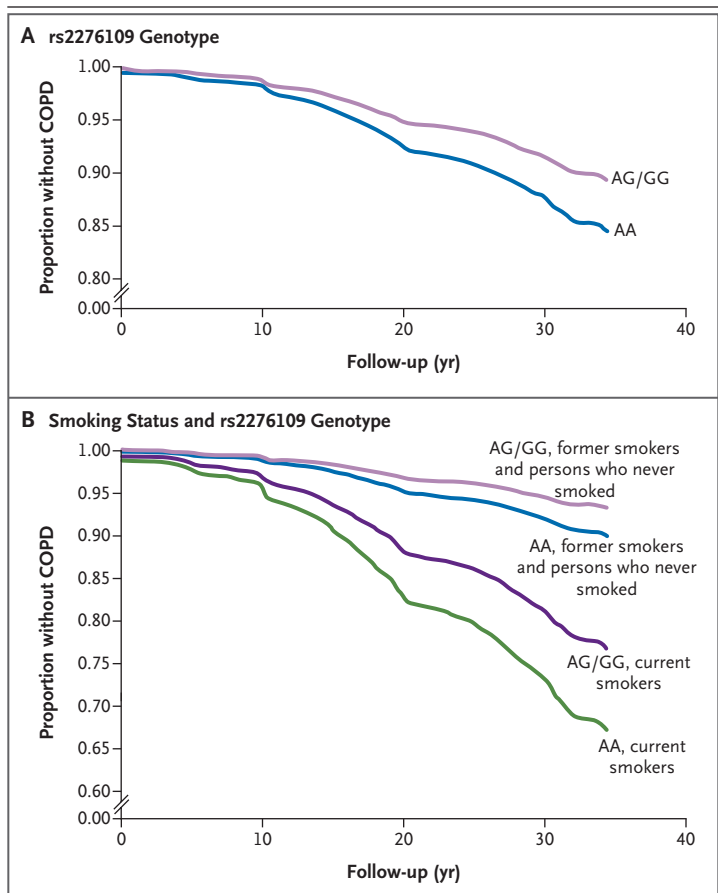
The population attributable risk of COPD due to homozygosity for the major allele of SNP rs2276109 was 28% (95% CI, 6 to 44). The addition of genotypic information on rs2276109 to a pooled logistic-regression model that included clinical variables did not result in improved discriminatory power for the risk of the onset of COPD ( $P=0.52$ ). We obtained similar results for SNP rs737693 (see Additional Results and Table 2 in the Supplementary Appendix).

##### eoCOPD and Lovelace

Given our findings with respect to COPD onset in the NAS cohort, we tested for an association between SNP rs2276109 and COPD in two additional cohorts. In this analysis, the minor allele of rs2276109 was significantly associated with a reduced risk of COPD in the eoCOPD and Lovelace cohorts (odds ratio in the Lovelace cohort, 0.63; 95% CI, 0.45 to 0.88) (Table 3).

##### Combined Analysis of COPD

We observed a significant association between the minor allele of SNP rs2276109 and a decreased risk of COPD in combined data from the NAS,



**Figure 2.** Proportion of Subjects without COPD, According to Genotype and According to Smoking Status and Genotype.

Estimated survival curves from Cox proportional-hazard regression models are shown for the Normative Aging Study (NAS) cohort. Panel A shows the estimated survival curves with subjects stratified according to the genotype for the single-nucleotide polymorphism (SNP) rs2276109 — subjects who were homozygous for the major allele of SNP rs2276109 and subjects who had at least one copy of the minor allele for SNP rs2276109. Panel B shows the estimated survival curves with subjects stratified according to current smoking status and genotype — subjects who were former smokers or who had never smoked and who were homozygous for the major allele of SNP rs2276109; subjects who were former smokers or who had never smoked and who had at least one copy of the minor allele for SNP rs2276109; current smokers who were homozygous for the major allele of SNP rs2276109; and current smokers with at least one copy of the minor allele for SNP rs2276109.

eoCOPD, and Lovelace cohorts (Table 3). This association remained significant in the analysis of the combined replication cohorts (i.e., after exclusion of data from the NAS cohort). We obtained similar results in a combined analysis of rs737693 and COPD in the NAS and eoCOPD cohorts (Table 2 in the Supplementary Appendix).

**Table 3. Results of Analyses of the Association between the A→G Minor Allele of SNP rs2276109 in the Promoter Region of *MMP12* and COPD in the NAS, eoCOPD, and Lovelace Cohorts.\***

| Association between rs2276109 and COPD, Expressed as P Values |        |          |  |  |
|---|--------|----------|--|--|
| NAS   | eoCOPD | Lovelace | Combined Overall†                        | Combined Replication‡                    |
| 0.02  | 0.006  | 0.005    | 4×10 <sup>-5</sup> (1×10 <sup>-5</sup> ) | 3×10 <sup>-4</sup> (2×10 <sup>-4</sup> ) |

\* The analysis included subjects in the longitudinal Normative Aging Study (NAS), families of subjects with severe chronic obstructive pulmonary disease (COPD) in the Boston Early-Onset COPD Study (eoCOPD), and subjects in the Lovelace Smokers Cohort (Lovelace). In each cohort, the association between the minor allele and the risk of COPD was in a negative direction. SNP denotes single-nucleotide polymorphism.

† Combined overall P values were calculated with the use of Fisher's method. The P value from a weighted z-score method is shown in parentheses.

‡ Combined replication P values were calculated with the use of Fisher's method after exclusion of data from NAS. The P value from a weighted z-score method is shown in parentheses.

## DISCUSSION

Most previous genetic association studies of lung function have included a single cohort; reported associations with respect to specific SNPs<sup>25,26</sup> and the direction of the associations<sup>27,28</sup> have been inconsistently replicated in subsequent studies. A strength of our study is that it included the analysis of multiple measurements of pulmonary function in a large number of subjects<sup>29</sup> — more than 20,000 FEV<sub>1</sub> measurements in more than 8300 subjects.

We found that the minor allele of a SNP in *MMP12* (rs2276109 [−82A→G]) was positively associated with lung function in children with asthma and in adult smokers with COPD or at risk for COPD. We also found that the minor allele of SNP rs2276109 was associated with a reduced risk of COPD in adult smokers. In the NAS cohort, the absence of the minor allele of rs2276109 was associated with a 54% increase in the risk of the onset of COPD and with a population attributable risk of COPD of 28%; the high frequency of the major (common) allele probably underlies this comparatively large risk.

The “Dutch hypothesis” states that asthma, chronic bronchitis, and emphysema are different manifestations of a disease entity that is influenced by host factors (e.g., genetic makeup) and exogenous factors (e.g., cigarette smoking).<sup>30,31</sup> This hypothesis is supported by our findings and those of others,<sup>26,32</sup> which suggest that certain genetic variants may play a role in the pathogenesis of both asthma and COPD.

*MMP12* is located on chromosome 11q22.3, in a cluster of genes encoding matrix metalloproteinases. The *MMP12* SNPs associated with increased FEV<sub>1</sub> are also associated with a delayed onset of COPD and a reduced risk of COPD. SNP rs2276109 is a functional polymorphism whose minor allele (G) has been associated with decreased promoter activity through less efficient binding of AP-1 in both murine and human monocytic cell lines.<sup>24</sup> Deletion of the AP-1 binding site abolishes both basal and stimulated expression of *MMP12*.<sup>33</sup> Thus, our findings are consistent with a beneficial role of reduced *MMP12* expression in pulmonary function and the pathogenesis of COPD in humans.

In the NAS cohort, the minor allele of rs2276109 was associated with increased FEV<sub>1</sub> but not with the rate of decline in lung function. Similarly, there was no association between two SNPs in *MMP12* and the rate of decline in lung function in the Lung Health Study (NCT00000568).<sup>34</sup> Although associations between haplotypes, including a SNP in *MMP12*, and a decline in lung function were reported in this study, the associations were inconsistent in direction and were not replicated in other cohorts.<sup>35</sup> Our results suggest that variants of *MMP12* are determinants of the level of lung function in subjects who are at risk for airflow obstruction (e.g., persons with asthma and smokers).

Our study has several limitations. First, we observed significant associations for rs2276109 in combined analyses, but these findings were not uniformly significant in each of the cohorts analyzed. This may be related to attenuated effects of this SNP in low-risk cohorts (e.g., those that included persons without asthma), false negative results due to small samples (e.g., in NETT<sup>35</sup>), or potential effects of medication (e.g., in CAMP).

Second, ascertainment bias may have negatively influenced our analysis of FEV<sub>1</sub> in CAMP and NETT, since both studies excluded subjects on the basis of increased disease severity (in the case of CAMP and NETT) or reduced disease severity (in the case of NETT), as defined according to FEV<sub>1</sub> measurements.<sup>11,14</sup> In addition to differences in geographic factors and ancestral history between the GACRS and CAMP cohorts, there may have been differences in exposure to allergens. Costa Ricans are exposed to high levels of dust-mite allergens,<sup>36</sup> a factor that



may be relevant, since exposure to dust-mite allergens can lead to the release of proinflammatory cytokines through an AP-1–dependent pathway.<sup>37</sup>

Third, our results for rs737693 and rs2276109 should be viewed as a single finding, since these SNPs are in tight linkage disequilibrium ( $r^2 > 90\%$ ) in all the cohorts that were analyzed. Although we suspect that our results are explained primarily by variation within the AP-1 binding site of the MMP12 promoter (rs2276109), variants in the 3′ genomic region of MMP12 (rs737693) could represent an alternative or additional explanation for our findings. Binding sites within a 3′ genomic region have been shown to influence the transcription of another matrix metalloproteinase.<sup>38</sup> Although we did not test SNPs in all genes adjacent to MMP12, our negative results for MMP3 and the linkage-disequilibrium patterns for the MMP12 region in the HapMap (Fig. 1 in the Supplementary Appendix) suggest that our results are due to variation in MMP12. There was no association between SNPs in linkage disequilibrium with rs2276109 and FEV<sub>1</sub> in adults in the British Birth Cohort, which is consistent with our negative findings with respect to an association between rs2276109 and FEV<sub>1</sub> in subjects without asthma and in persons who never smoked. However, additional findings of an association between MMP12 SNPs and FEV<sub>1</sub> in the British Birth Cohort suggest that there could be additional susceptibility variants for lung function in MMP12.<sup>39</sup>

Finally, caution should be exercised in interpreting the population attributable risks from our analyses. Although our findings strongly suggest that MMP-12 has a role in determining lung function and susceptibility to COPD in high-risk groups, knowledge of the genotype for an MMP12 variant does not add to clinical variables in predicting the onset of COPD (which is consistent with previous findings with respect to the predictive capacity of replicated genetic associations).<sup>40</sup>

In summary, our findings suggest that the minor allele of SNP rs2276109 in MMP12 is associated with lung function in children with asthma and adult smokers, as well as with the risk of COPD in adult smokers.

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