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Variants of asthma and chronic obstructive pulmonary disease genes and lung function decline in aging

Audrey. H. Poon, PhD[1,2,3], E. Andres Houseman, DSc[4], Louise. Ryan, PhD[4]*, David.Sparrow, DSc[5], Pantel.S. Vokonas, MD[5], Augusto.A. Litonjua, MD,MPH [1,2,6].

[1]Channing Division of Network Medicine, Brigham and Women's Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA; [3]Meakins Christie Laboratories, McGill University Health Centre (MUHC), Montreal, Quebec, Canada; [4]Harvard School of Public Health, Boston, MA; [5]Normative Aging Study, VA Boston Healthcare System and Department of Medicine at Boston University School of Medicine, Boston, MA; [6]Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Boston, MA;

*Current Affiliation: Commonwealth Scientic and Industrial Research Organisation (CSIRO), Division of Mathematics, Informatics and Statistics (CMIS), North Ryde NSW 1670, AUSTRALIA

Corresponding author
Augusto A. Litonjua, MD, MPH
Channing Division of Network Medicine
Brigham & Women's Hospital
Phone: (617) 525 – 0997
Fax: (617) 525 – 0958
e-mail: augusto.litonjua@channing.harvard.edu

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ABSTRACT

Background: A substantial proportion of the general population has low lung function, and lung function is known to decrease as we age. Low lung function is a feature of several pulmonary disorders, such as uncontrolled asthma and chronic obstructive pulmonary disease (COPD). The objective of this study is to investigate the association of polymorphisms in asthma and COPD candidate genes with rates of lung function decline in a general population sample of aging men.

Methods: We analyzed data from a cohort of 1047 Caucasian men without known lung disease, who had a mean of 25 years of lung function data, and on whom DNA was available. The cohort was divided into 2 groups, and we tested a total of 942 SNPs in 45 asthma and COPD candidate genes in the first group (testing cohort, n=545) for association with change in FEV_1 over time.

Results: One hundred nineteen SNPs that showed nominal associations in the testing cohort were then genotyped and tested in the second group (replication cohort, n=502). Evidence for association from the testing and replication cohorts were combined, and after adjustment for multiple testing, 13 variants of 7 genes (*DPP10, NPRS1,SFTPD, VDR/COL2A1, TGFB1/CCDC97,MMP12 and ADAM33)* remained significantly associated with change in FEV_1 over time.

Conclusions: Our findings that genetic variants of genes involved in asthma and COPD are associated with lung function decline in normal aging subjects suggest that similar genetic mechanisms may underlie lung function decline in both disease and normal aging processes.

INTRODUCTION

For healthy non-smokers, the forced expiratory volume in 1 second (FEV_1), increases from birth and reaches its peak at around the ages of 20 and 25 years (the growth phase), it remains stable until the ages of 30 to 35 years (the plateau phase), and begins to decline with aging (the decline phase)(1-3). In the elderly, low lung function is associated with impaired cognitive function, reduced physical activity and all-cause mortality(4-6). Since a substantial proportion of the general population has unrecognized low lung function, its impact on health and quality of life can easily be underestimated(7).

Segregation studies have suggested a genetic contribution to lung function variability in the general population(8, 9). Linkage(10-12) and association(13-15) studies further attempted to localize the genetic loci influencing lung function. Since low lung function is a feature of uncontrolled asthma and COPD, we hypothesized that genetic variants that predispose to asthma and COPD might underlie the rapidity of lung function decline. We conducted a longitudinal lung function study in a cohort of men with available DNA and over 25 years of lung function data to determine if polymorphisms of asthma and COPD candidate genes are determinants of lung function decline in a healthy aging population.

METHODS

The study cohort was a subset of the original Normative Aging Study (NAS) cohort(16), a longitudinal study of aging established by the Veterans Associations (VA), with recruitment between 1961-1970. A total of 1245 men had lung function data and adequate DNA samples at the time of the study. We removed 198 subjects who developed asthma, emphysema or chronic bronchitis after entry into the cohort, for a total of 1047 men included in this analysis. Details are provided in the Supplementary Material. The study protocol was approved by the Human Studies Subcommittee of the Department of Veterans Affairs Medical Center and the Institutional Review Board of the Brigham and Women's Hospital.

Lung function was measured in a standardized manner beginning in 1963.(17) Beginning in 1984, a new spirometer was used along with new standardized protocols that adhered to American Thoracic Society (ATS) standards for pulmonary function measurement,(18) and these protocols were updated subsequently(19, 20). Further details are provided in the Supplementary Material. The phenotype of interest was lung function decline defined as the change in forced expiratory volume in 1 second (FEV_1) between two consecutive visits over the number of years between the two visits:

$$\Delta FEV_1 = (FEV_{1 \text{ at visit } n+1} - FEV_{1 \text{ at visit } n}) / (\text{age}_{\text{ at visit } n+1} - \text{age}_{\text{ at visit } n}),$$

where n is visit number ($1 \leq n \leq 13$).

Candidate genes were selected based on their known or suspected roles in the pathogeneses of asthma and COPD, from review of the existing literature performed by 2 of the authors (AHP and AAL). In particular, they are genes which have been identified to be asthma or asthma-related phenotype genes through positional cloning or

candidate gene association testing. SNPs in 19 candidate genes were selected for investigation if they were either (1) tagging SNPs with $r^2 < 0.80$ and minor allele frequency > 5%, covering 5kb upstream and downstream of the first and last exons of each gene, (2) non-synonymous amino acid change with minor allele frequency > 1% or (3) known associated variants with asthma, COPD, and related phenotypes.

Genotyping of SNPs for the screening cohort was carried out using the Illumina BeadStation 500G (San Diego, CA, USA). Genotyping for the replication cohort was carried out using one of two platforms, the Sequenom MassArray MALDI-TOF mass spectrometer (Sequenom, CA, USA) and the TaqMan 5' exonuclease assays (Applied Biosystems, CA, USA)(21). Details of genotyping are provided in the Supplementary Material.

A two-stage testing - replication strategy was adopted where the study population was divided into 2 subsets: a testing cohort (TC) and a replication cohort (RC). The entire SNP set was tested for associations between individual SNP and lung function decline in TC in the presence of potential confounders (height, age, smoking status, and intensity of cigarette smoking in pack-years). Mixed models were implemented in the Mixed Procedure in SAS (SAS Institute, Inc., Cary, NC), using the "Repeated" statement to account for correlations between repeated observations on each subject. Further details of statistical methods can be found in the Supplementary Material. SNPs associated in the TC at $p \leq 0.1$ under either additive or recessive models were genotyped in RC. Associated SNPs were tested by the same statistical method and under the same genetic model as those observed in TC. Analyses in RC were constrained to having the same direction of effect as in TC, thus one-sided testing was

employed in the RC. Due to the consistency in direction between associations in TC and RC, two-sided P-values from TC and one-sided P-values from RC were combined using Fisher's method(22). Bonferroni correction was applied to adjust the p-values. Population stratification was assessed using PLINK(see Supplementary material)(23).

RESULTS

Population characteristics

The study sample consisted of 1047 subjects, who had DNA samples, smoking history and lung function data, and were randomly divided into 545 and 502 subjects for TC and RC, respectively. The two cohorts were comparable in demographic, lung function and smoking characteristics (Table 1). At baseline, the mean age was 41.3 years (standard deviation (sd) = 8.2) in the TC, and was 41.0 years (sd=7.8) in the RC. The mean FEV_1 values as percent predicted were 97.51% (sd=12.0) and 96.7% (sd=11.0) in the TC and RC, respectively. At baseline, the proportions of never, current, and former smokers were also comparable in both cohorts. The mean number of follow-up visits were 8.5 (sd=2.3) in the TC and 8.6 (sd=2.2) visits in the RC, with a mean of 24.42 yrs (± 6.70) of follow-up for the TC and 25.42yrs (± 6.20) of follow-up in the RC.

SNP Associations

A total of 943 SNPs from 44 candidate genes were genotyped and analyzed in the TC under both additive and recessive models (see Supplementary Material, Table S1). Genotyping success rates of > 90% were achieved for all but 13 SNPs. These 13 SNPs were excluded from subsequent analyses. A total of 194 SNPs were associated with change in FEV_1 at p-value ≤ 0.1. Twenty-four of the 194 SNPs were associated under both additive and recessive models, and the association under the recessive model for all the 24 SNPs were more statistically significant, hence for these 24 SNPs, only the recessive model was tested in the RC. After removal of 11 SNPs that were out

of Hardy Weinberg equilibrium, a total of 119 SNPs were successfully genotyped and analyzed in the replication cohort. Forty-four SNPs were found to have the same direction of effect in both the TC and RC, and evidence for association was combined. A total of 13 SNPs remained statistically significant for their association with decline in FEV_1, after Bonferroni adjustment (Table S4).

Table 2 presents the results of the mixed effects modeling of lung function decline. Under a recessive model, 4 variants of *DPP10* (rs17783638, rs958457, rs4849383 and rs4849384), 4 variants of *NPSR1* (rs323917, rs725902, rs17170012 and rs11771425), a variant of *MMP12* (rs17099726), a variant of *VDR* (rs12368284) and a variant of *ADAM33* (rs543749) were associated with rates of lung function decline. Homozygosity for the minor alleles of *DPP10, NPSR1* and *ADAM33* variants conferred a slower rate of FEV_1 decline compared with carriers of the major allele (also see Supplemetary Material). Under an additive model, the presence of each additional minor allele of variants rs7078012 of *SFTPD* and rs10417924 of *TGFB1* were associated with faster rates of FEV_1 decline.

In addition, the same variants were also tested for association with rates of FVC decline (Table 3), defined in a similar way as for FEV_1 decline. Five of the 13 FEV_1 decline associated SNPs (rs177836, rs958457 and rs484938 of *DPP10* and rs117714 of *NPSR1* and rs543749 of *ADAM33*) were associated with rate of FVC decline ($p<0.05$). Variants of DPP10 and ADAM33 were significantly associated with rate of FVC decline in the same direction as that observed for rate of FEV_1 decline where homozygotes of the rare allele confer a slower rate of FEV_1 and FVC_1 decline. On the contrary, variant rs117714 of *NPRS1* was significantly associated with both FEV_1 and

FVC decline, but in the opposite direction. The rare homozygote of *NPRS1* rs117714 confers a slower rate of FEV_1 decline but the same genotype confers a faster rate of decline in FVC.

Discussion

We conducted a candidate gene analysis and report genetic variants influencing lung function decline in a general population sample of men that was initially recruited to study healthy aging. Findings of genetic association with lung function decline in general populations have been reported using longitudinal data(14, 15), yet the number of candidate genes and their SNPs investigated were limited. To date, only one genome-wide study of lung function decline has been published and that study had no findings that reached genome-wide statistical significance(24). However, in that study, the longest mean follow-up time was only 14.6 yrs (±7.2 yrs), and most of the cohorts only had 2 lung function measures. The strength of this study derives from the availability of multiple, repeated lung function and predictor information gathered over a mean of 25 years, allowing us to account for changes in exposures (i.e. age and smoking) over time.

We found *DPP10*, *NPRS1*, *VDR* and *ADAM33* to be associated with lung function decline and these genes have all been shown to be associated with asthma in multiple populations; *ADAM33* was implicated as an asthma gene in 17 populations, *DPP10* in 4 *VDR* in 6 and *GPR154* in 9, as summarized in Michel et al(25). In our study, the associated variant *ADAM33* rs542749, also known as V-1, has been found to be associated with asthma(26) and atopy(27). Furthermore, rs543749 has been found to be associated with COPD, FEV_1 percent predicted, FEV_1/FVC and FEF_{25-75} percent predicted in chronic smokers(28). In the chronic smoker cohort(28), individuals with the CC genotype have significantly lower lung function (FEV_1 percent predicted, FEV_1/FVC and FEF_{25-75} percent predicted), in line with our findings that the AA genotype confers a

slower rate of FEV_1 decline (protective effect). The functional consequence of rs543749 is unknown. It is intronic and in high linkage disequilibrium with a nearby block housing the isoluecine → valine variant rs3918396. Unfortunately variant rs3918396 was not genotyped due to technical reason. Expression of ADAM33 protein in structural cells of the airways(29) and associations between variants of *ADAM33* and impaired early lung-function, lung morphogenesis, lung function decline suggest a role of this protein in airway remodeling(15, 28).

The 4 associated variants of *DPP10*, which encodes dipeptidyl peptidase X, all reside between exons 1 and 2, an area where alternate splicing occurs to encode multiple isoforms of different length and where association with asthma has been observed(30). Furthermore, a variant located in intron 1 (rs13011555) has also been found to be associated with FEV_1 in Caucasian adults (The British 1958 Birth Cohort)(31). The significant yet small effect sizes estimated in our study and the British 1958 Birth Cohort suggest that *DPP10* is one of the many genes influencing lung function. However, the mechanism remains unknown.

In addition to *ADAM33* and *DPP10*, neuropeptide S receptor 1 (*NPRS1*), also known as G-protein receptor 154 (*GPR154*) is the third asthma gene discovered through positional cloning and found to be associated with rates of FEV_1 decline in our study. NPRS1 has been shown, when activated by its endogeneous agonist neuropeptide S, to increase cAMP and Ca^{2+} levels(32) and its mRNA expression profile suggests a role in modulating macrophage and eosinophils immune responses(33). Furthermore, mRNA expression of NPRS1 has been found to be elevated in the ciliated cells of the airway epithelium of asthmatic subjects compared to controls, suggesting a

role of NPRS1 in airway defense(34).. Polymorphisms of *NPRS1* have been found to be associated with asthma or related phenotypes (e.g. IgE, airway responsiveness) in several populations(34-36). A total of 4 variants were found to be associated with rates of FEV_1 decline in this study. Homozygotes of the rare alleles for all variants confer a slower rate of FEV_1 decline. Of the 4 associated variants, rs323917 has previously been shown to be associated with airway hyperresponsiveness, with the rare allele associated with increased airway hyperresponsiveness(35). Immunocytochemistry staining of bronchial biopsy tissue of asthmatic and non-asthmatic subjects has shown that NPRS1 protein is expressed in bronchial epithelium cells of asthmatic and not in control subjects(37). Furthermore, the expression of tenascin C mRNA has been shown to be regulated by NPS, the ligand of NPSR1, suggesting that NPRS1 may mediate lung function via tenascin C, an extracellular matrix protein expressed during inflammation(37).

One variant of *SFTPD*, *MMP12,* VDR and *TGFB1* have been found to be associated with rate of FEV_1 decline in our study. Genetic variants of *SFTPD* and *MMP12* have been found to be associated with COPD, lung function and/or asthma(38). The minor allele (T) of variant rs7078012 of *SFTPD* was associated with a faster rate of FEV_1 decline in our study, and the same allele was observed to be associated with two cohorts of COPD(39), with a protective effect. Variant rs17099726 resides around 6kb upstream of *MMP12* and upstream of the functional variant rs2276109, which has been shown to be associated with lung function in asthmatic children and adults who smoke; and with development of COPD in the NAS population(38). However, in the general population of non-COPD subjects, the functional variant rs2276109 was not associated

with lung function decline. At present, the biological function of rs17099726 is unknown. Noteworthy is the location of the associated variants of *VDR* and *TGFB1*, which are mapped onto intronic region of nearby genes. Variant rs12368284 is located upstream of exon 1f of *VDR* and in an intronic region towards the 3' end of the collagen type II, alpha 1 (*COL2A1)* gene; similarly variant rs10417924 resides in a LD block spanning between 3' end of *TGFB1* and the entire coiled-coil domain containing 97 (*CCDC97)* gene. Given the gene-level replications observed between variants of *TGFB1* and COPD, the effect of this variant is likely mediated through TGFB1(40).

In addition to rates of FEV_1 decline, 6 of the 13 variants also showed significant association with rates of FVC decline, suggesting that the mechanisms which *DPP10*, *NPRS1* and *ADAM33* operated under affect rates of lung function decline potentially through both airway caliber and lung volume.

This study has its limitations. Since the cohort is composed of Caucasian men, associations detected in this cohort may not be generalizable to women and non-Caucasians. In addition, since the mean age of this cohort at baseline was 41 years, genetic factors which we have identified to influence lung function decline may not be the same as those that influence growth in younger populations. Because genotyping for this project began several years ago, we were unable to include variants from genes that have recently been associated with asthma (e.g. *ORMDL3, PDE4D*), COPD (*CHRNA 3/5*), or lung function (*GSTO2 and IL6R*). Nevertheless, our findings that genetic variants of genes involved in asthma and COPD pathogenesis are associated with lung function decline in normal aging subjects suggest that similar genetic mechanisms underlie lung function decline in both disease and normal aging processes.

While longitudinal studies are generally thought to provide more accurate estimates of lung function decline, these types of studies also have problems such as learning effects, loss to follow-up, variability over time of spirometers and technicians. We have attempted to minimize these effects where possible. While spirometry was performed in a standardized manner from the inception of the study in the 1960s(17), standardization was modified beginning in 1984 to comply with the recommendations from the ATS(18), and a different spirometer was used. We attempted to account for this change by creating a variable that identified the method and adjusted this in our analyses. While the standardization method itself was a significant determinant of lung function decline, it did not affect the observed associations between the SNPs and lung function decline, whether the variable was in the model or not. Additionally, we used standardized protocols for measuring spirometry to minimize variability between technicians.

In summary, we have found that variants in 7 asthma and COPD candidate genes were determinants of lung function decline over 30 years in a cohort of men. Our results suggest that mechanisms involved in the development of asthma and COPD are operating in the normal process of decline in lung function seen with aging.

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U.S. Department of Veterans Affairs, and is a component of the Massachusetts Veterans Epidemiology Research and Information Center, Boston, Massachusetts.

Table 1: Baseline population characteristics

	Testing cohort	Replication cohort
Number of subjects	545	502
Age (yrs), mean (sd)	41.3 (8.2)	41.1 (7.8)
Age range (yrs)	23.1-70.1	23.4-65.4
FEV_1% predicted, mean (sd)	97.5 (12.0)	96.7 (11.0)
FEV_1 (L), mean (sd)	4.0 (0.6)	4.0 (0.6)
Smoking Status (%)		
Nonsmoker	37.2	31.0
Current smoker	30.4	35.5
Former smoker	32.3	33.5
Number of follow up visits (N), mean (sd)	7.4 (2.3)	7.7 (2.2)
Number of years of follow-up (yrs), mean (sd)	24.64 (6.70)	25.42 (6.20)

*mean value, yrs = years, sd = standard deviation, L – litre, ml = milliliter.

Table 2: Effect size of associated SNPs and FEV_1 decline

Gene	SNP	Genetic Model*	Genotype	Effect estimate in TC (SE)†	Effect estimate in RC (SE) †	combined p-value
DPP10	RS17783638	recessive	CC	11.96 (1.63)	2.80 (5.80)	$2.86x10^{-12}$
DPP10	RS958457	recessive	GG	5.36 (3.01)	6.09 (2.80)	0.0086
DPP10	RS4849383	recessive	GG	4.93 (2.09)	11.94 (1.81)	$1.25x10^{-11}$
DPP10	RS4849384	recessive	CC	17.93 (5.56)	16.62 (2.56)	$2.19x10^{-12}$
NPRS1	RS323917	recessive	GG	16.58 (1.79)	10.48 (1.86)	$1.860x10^{-26}$
NPRS1	RS725902	recessive	GG	7.03 (2.08)	1.78 (4.80)	0.0024
NPRS1	RS17170012	recessive	GG	22.85 (2.84)	4.71 (2.68)	$2.07x10^{-15}$
NPRS1	RS11771425	recessive	GG	9.76 (1.57)	4.57 (3.84)	$1.52x10^{-9}$
SFTPD	RS7078012	additive	T	-4.97 (1.60)	-1.44 (1.60)	0.0031
MMP12	RS17099726	recessive	GG	-14.94 (3.86)	-25.73 (17.98)	$1.08x10^{-4}$
VDR/COL2A1	RS12368284	recessive	GG	-9.14 (2.97)	-0.08 (2.14)	0.0082
TGFB1/CCDC97	RS10417924	additive	T	-3.19 (1.87)	-3.74 (1.46)	0.004
ADAM33	RS543749	recessive	TT	9.62 (3.28)	4.75 (6.00)	0.006

* Genetic model refers to the coding of alleles in the mixed models, as explained in the Methods section.

† Effect estimates obtained from mixed effects models. Effect estimates are displayed as ml/year change in FEV_1; reference group = homozygotes plus heterozygotes of the common allele (recessive model). For additive models. effect estimates are ml/year change in FEV_1 for each additional minor allele displayed, compared with the presence of the major allele. Positive estimates denote slower decline associated with the genotype, while negative estimates denote faster decline.

Table 3: Effect size of associated SNPs and FVC decline

Gene	SNP	Genetic Model*	Genotype	Effect estimate in TC (SE)†	Effect estimate in RC (SE) †	combined p-value
DPP10	RS17783638	recessive	CC	16.37 (3.59)	5.63 (4.13)	4.22×10^{-6}
DPP10	RS958457	recessive	GG	5.93 (3.57)	5.64 (3.86)	0.04
DPP10	RS4849383	recessive	GG	10.52 (1.84)	27.88 (2.29)	5.90×10^{-40}
NPRS1	RS11771425	recessive	GG	-9.36 (2.42)	-14.08 (11.68)	8.45×10^{-5}
ADAM33	RS543749	recessive	TT	11.09 (4.95)	8.11 (7.34)	0.02

* Genetic model refers to the coding of alleles in the mixed models, as explained in the Methods section.

† Effect estimates obtained from mixed effects models. Effect estimates are displayed as ml/year change in FEV_1; reference group = homozygotes plus heterozygotes of the common allele (recessive model). Positive estimates denote slower decline associated with the genotype, while negative estimates denote faster decline.

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