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Genetic variation in antioxidant enzymes, cigarette smoking, and longitudinal change in lung function

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ABSTRACT

Antioxidant enzymes play an important role in the defense against oxidative stress in the lung and in the pathogenesis of chronic obstructive pulmonary disease (COPD). Sequence variation in genes encoding antioxidant enzymes may alter susceptibility to COPD by affecting longitudinal change in lung function in adults. We genotyped 384 sequence variants in 56 candidate genes in 1281 African American and 1794 European American elderly adults in the Health, Aging, and Body Composition study. Single-marker associations and gene-by-smoking interactions with rate of change in FEV_1 and FEV_1/FVC were evaluated using linear mixed-effects models, stratified by race/ethnicity. In European Americans, rs17883901 in *GCLC* was statistically significantly associated with rate of change in FEV_1/FVC; the recessive genotype (TT) was associated with a 0.9% per year steeper decline ($P = 4.50 \times 10^{-5}$). Statistically significant gene-by-smoking interactions were observed for variants in two genes in European Americans: the minor allele of rs2297765 in *mGST3* attenuated the accelerated decline in FEV_1/FVC in smokers by 0.45% per year ($P = 1.13 \times 10^{-4}$); for participants with greater baseline smoking pack-years, the minor allele of rs2073192 in *IDH3B* was associated with an accelerated decline in FEV_1/FVC ($P = 2.10 \times 10^{-4}$). For both genes, nominally significant interactions ($P < 0.01$) were observed at the gene level in African Americans ($P = 0.007$ and 4.60×10^{-4}, respectively). Nominally significant evidence of association was observed for variants in *SOD3* and *GLRX2* in multiple analyses. This study identifies two novel genes associated with longitudinal lung function phenotypes in both African and European Americans and confirms a prior finding for *GCLC*. These findings suggest novel mechanisms and molecular targets for future research and advance the understanding of genetic determinants of lung function and COPD risk.

Lung function is an important predictor of morbidity and mortality in the general population [1]. Spirometric measures of lung function, such as forced expiratory volume in the first second (FEV_1) and the ratio of FEV_1/forced vital capacity (FVC) are easily measured and reliable indicators of the physiological state of the lungs and airways and provide the basis for diagnosing and staging chronic obstructive pulmonary disease (COPD) [2]. Decline in lung function occurs naturally with aging, but accelerated decline can be caused by exposures such as cigarette smoking and can lead to low lung function that characterizes COPD [3,4]. Therefore, longitudinal changes in lung function are informative predictors of COPD risk, and studies of these outcomes provide important insights for understanding disease pathogenesis [3–6].

The imbalance between chronic oxidative stress and antioxidant protection is postulated to play a key role in accelerated lung

Abbreviations: COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; GCLC, glutamate–cysteine ligase (catalytic subunit); GGT2, γ-glutamyl transferase 2; GLRX, glutaredoxin; GST, glutathione *S*-transferase; IDH, isocitrate dehydrogenase; mGST, microsomal glutathione *S*-transferase; SOD, superoxide dismutase

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function loss [7,8]. Cigarette smoke, a major source of exogenous oxidants, exposes the lung to elevated levels of oxidative stress, whereas dietary antioxidants and endogenous antioxidant enzymes are the two major forms of antioxidant defense that counteract these processes. The observation that only a subset of smokers develop COPD [9] and that a substantial proportion of COPD cases cannot be explained by smoking [10] led to the hypothesis that dietary intake of antioxidants and genetic variation in genes encoding antioxidant enzymes both play an important role in modifying antioxidant defense against cigarette smoke in the lung with ultimate effects on COPD risk.

In support of this hypothesis, observational epidemiologic studies have provided evidence of a positive association between dietary antioxidant intake and lung function, with stronger effects in cigarette smokers [11–15]. Genetic variation in antioxidant enzymes has also been studied in candidate gene association studies using population data, but published studies have limitations [16]. First, most studies considered only limited numbers of candidate genes, leaving many biologically relevant genes unstudied. Second, very few studies considered longitudinal lung function phenotypes [17–19]. Third, despite compelling evidence for their importance [20,21], gene-by-smoking interactions are rarely investigated. Finally, very few studies include individuals of non-European ancestry, limiting inference to individuals of European descent. Although recent, large-scale genome-wide association studies (GWAS) of lung function phenotypes together identified numerous novel genetic loci, these studies are limited in that they consider only European ancestry and cross-sectional phenotypes [22–24].

We hypothesized that common single-nucleotide polymorphisms (SNPs) in genes encoding antioxidant enzymes affect longitudinal decline in lung function. We further hypothesized that gene-by-smoking interactions are present such that some genetic variants affect lung function decline contingent on exposure to cigarette smoke. To investigate these hypotheses, we selected 56 candidate genes that either had putative functional relevance to antioxidant defense in the lung or were previously investigated in relation to COPD-related phenotypes. Functional and tagging SNPs in these genes were genotyped and tested for single-marker associations and gene-by-smoking interactions with rate of change in FEV_1 and FEV_1/FVC in a population of African American and European American elderly adults from the Health, Aging, and Body Composition (Health ABC) Study.

Material and methods

Subjects

The Health ABC study is a longitudinal, prospective cohort study comprising 1281 African American and 1794 European American community-dwelling men and women, ages 70–79 years at baseline (1996–1997) and residing in the metropolitan areas of Pittsburgh, Pennsylvania, and Memphis, Tennessee [25]. Participants reported self-proclaimed race initially as "black" or "white," but the terms "African American" and "European American" are used herein. To be eligible, participants were required to be ambulatory at baseline as confirmed by self-report of no difficulty walking one-quarter of a mile or climbing 10 steps without resting, no difficulty performing basic activities of daily living, and no use of a cane, walker, crutches, or other special equipment to ambulate. In addition, participants were required to have no history of active treatment for cancer in the prior 3 years and no plan to move out of the area in the subsequent 3 years. The Health ABC study was approved by the Institutional Review Boards of the University of Pittsburgh and the University of Tennessee, and the work reported herein was approved by the Institutional Review Board for Human Participants at Cornell University.

Pulmonary function testing

Spirometry was completed at four time points (baseline and years 4, 7, and 9) in accordance with standardized guidelines of the American Thoracic Society (ATS), as previously reported [25]. The study used a horizontal, dry rolling seal HF6 spirometer (Sensor Medics Corp., Yorba Linda, CA, USA) during clinical visits and the EasyOne Model 2001 diagnostic spirometer (ndd Medizintechnik AG, Zurich, Switzerland) during home visits starting in year 8. The two devices were evaluated for comparability and provided virtually identical values. Consistent with the quality control standard used in recent lung function GWAS [22–24], all FEV_1 (ml) and FEV_1/FVC (%) measures meeting the ATS criteria for acceptability were included in this study.

Cigarette smoking

Participants were classified based on their long-term smoking status during the study follow-up as: (1) never smokers (never smoker at all spirometry time points), who were considered as the reference group in analyses; (2) persistent smokers (current smoker at all time points); (3) former smokers (former smoker at all time points); and (4) intermittent smokers (changing smoking status at different time points). Lifetime smoking dose was quantified as pack-years and calculated at study baseline for current and former smokers.

Candidate gene selection and genotyping

Based on a previous systematic review of genetic association studies and gene expression studies investigating antioxidant enzymes and COPD-related phenotypes [16], we identified 56 candidate genes encoding antioxidant enzymes known to be expressed in lung tissue and postulated to affect the balance of antioxidants/oxidants. Three hundred and eighty-four functional and tagging SNPs were selected to capture variation across each gene and its regulatory regions (2 kb upstream and downstream). Details of the SNP selection strategy are provided elsewhere [26]. Separate consideration was given to African Americans and European Americans in SNP selection to maximize coverage in both populations, given differences in linkage disequilibrium (LD) structure and allele frequencies. Details of DNA extraction and genotyping quality, which were excellent, are provided elsewhere [26].

Four genes (*GGT2, GSTK1, GSTM1,* and *GSTT1*) were excluded from subsequent analyses because of low genotyping quality or atypical clustering of assayed SNPs. For the remaining SNPs with successful genotyping, Hardy–Weinberg equilibrium (HWE) was tested using the χ^2 goodness-of-fit test, stratified by race/ethnicity. After removing SNPs with genotyping call rate $< 95\%$, minor allele frequency (MAF) $< 1\%$, or $P < 0.005$ for the HWE test, the study included 314 SNPs in 52 genes in the African American analyses and 284 SNPs in the same 52 genes in the European American analyses (Supplementary Table 1).

Statistical analysis

Linear mixed-effects models were used to investigate single-marker associations and gene-by-smoking interactions with rate of change in FEV_1 and rate of change in FEV_1/FVC; all analyses were stratified by race/ethnicity. A continuous time variable quantified the time elapsed between each spirometry test and the study baseline. Random intercept and time effects were