NUTRITIONAL GENOMICS OF PULMONARY FUNCTION

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Accelerated pulmonary function decline is a key risk factor for development of chronic obstructive pulmonary disease (COPD), the 3rd leading cause of death worldwide. Chronic inflammation is central to the pathogenesis of steep pulmonary function decline and COPD and a healthy diet rich in sources of antioxidant and anti-inflammatory nutrients is hypothesized to counter inflammation and contribute to better lung function and lower risk of COPD. This dissertation investigated whether higher levels of biomarkers of nutrients with anti-inflammatory function (i.e., vitamin D, omega-3 fatty acids) and nutrient with antioxidant function (i.e., vitamin E) were associated with higher pulmonary function and whether the nutrients' positive effects were stronger in cigarette smokers, given the oxidative damage smoking causes in the lungs. In addition, given the role of genetic variation in nutrient metabolism and pulmonary function, this dissertation also investigated whether nutritional status and genetic variation jointly affect pulmonary function.

First, we designed a cohort-specific analysis plan and conducted a meta-analysis in 8 cohort studies to investigate the cross-sectional association of serum vitamin D concentration with pulmonary function (measured by forced expiratory volume in the 1st second [FEV_1] and forced vital capacity [FVC]). We found consistently positive associations across the cohorts and the ancestries (European ancestry [EA] and African ancestry [AA]), with a stronger effect in ever smokers.

Next, we investigated another set of nutrients that contribute to anti-

inflammatory pathways, specifically the omega-3 fatty acids. We conducted a meta-analysis in 7 cohorts to investigate whether the association of genetic variants with pulmonary function depends on blood levels of omega-3 fatty acids. We found that docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) were positively associated with pulmonary function, with a stronger effect in current smokers. In the genome-wide interaction analysis, we identified a novel genetic variant, the major allele (A) of rs11683320 on the *DPP10* gene that was inversely associated with FVC. The negative association of rs11683320 was attenuated by a higher DHA concentration, with a stronger effect in former smokers. This novel locus, which was missed in previous standard genome-wide association studies (GWAS) that did not account for the interaction with DHA, was replicated in one independent cohort study and passed the Bonferroni-corrected genome-wide significance level ($P < 1.25 \times 10^{-8}$).

In the third study, we analyzed data from a completed randomized controlled trial to conduct an 'as-treated' analysis of vitamin E supplementation on FEV_1 decline in healthy males over 50 years old. Greater increase in plasma vitamin E over 3 years was associated with slower FEV_1 decline, with evidence of effect modification by smoking. We also found that the minor allele (T) of a missense mutation (frequency = 31% in EA and 13% in AA), rs2108622, on the *CYP4F2* gene, which was identified in a previous GWAS of plasma response to vitamin E supplementation in heavy male smokers, was associated with greater increase in plasma vitamin E after supplementation in our study of healthy men.

This dissertation research used novel methods, including a gene-by-nutrient interaction study of large multi-ethnic cohort studies to contribute to the knowledge base on nutrition and lung health. Ultimately, such information is needed to understand whether population subgroups, for example as defined by genetic variation and/or by smoking status, might benefit differentially from dietary interventions.

BIOGRAPHICAL SKETCH

Jiayi Xu was born on June 28, 1992, the only child of her parents, Weiding Xu and Donglan Xie. She grew up in Shanghai, China and lived with her parents and grandparents before going to a junior boarding school. From a young age, Jiayi enjoyed reading science books and watching the Discovery Channel. She always had an innate curiosity with the world and an appreciation for its complexity and beauty. Since then, Jiayi has explored her curiosity about the world in many different ways, from brainstorming ideas to create a "greener" planet as an elementary schooler, learning the basic principles of chemical reactions in junior high school, designing her own experiments in high school, to studying abroad as a college student majoring in nutrition.

Jiayi spent the first half of her college life at Shanghai Jiao Tong University and the second half at Cornell University, achieving a Bachelor of Science in Nutritional Sciences with a concentration in dietetics and distinction in research. As a senior undergraduate, Jiayi was awarded the Academic Excellence Award for achieving the highest GPA within her major in her college. In the midst of her undergraduate honor thesis project, conducted under the supervision of Dr. Christine Olson, Jiayi became intrigued with the systematic and rigorous approach utilized in the field of human nutrition to answer unknown questions in the world, and to contribute to creating a better life for humankind.

After graduating from college, Jiayi further pursued her research interest in the nutrition field and stayed at Cornell University for her PhD program in human nutrition. Her training in nutrition as well as her interests in public health encouraged her to reach out to Dr. Patricia Cassano, who had profound knowledge and expertise in nutritional epidemiology and later became Jiayi's PhD dissertation advisor. After

joining Dr. Cassano's group, Jiayi began to hone her research on the interaction between genetics (internal) and nutrients (external) as well as their impact on individual health, and decided to minor in Genomics and Epidemiology to better equip herself to study this topic. During her PhD program, Jiayi was awarded the distinction of Genomics Scholar by the Cornell University Center for Vertebrate Genomics and received a Pfizer Predoctoral Fellowship from the American Society of Nutrition for her dissertation work. Jiayi was the first author on two papers by the end of her fourth year. The first paper was published in the *British Journal of Nutrition* and the second paper in the *American Journal of Respiratory and Critical Care Medicine* with an accompanied editorial article. Jiayi is currently working on her last dissertation project, which has a special place in her heart because she led the project cradle-to-grave, from the initial genotyping grant application submitted to the National Heart, Lung, and Blood Institute, to the final write-up of the paper manuscript. Jiayi was able to accomplish all of this due in large part to the invaluable mentorship she received from her advisor, Dr. Cassano, as well as the co-PI, Dr. Dana Hancock at Research Triangle Institute.

After completing her PhD program, Jiayi is about to embark on her next adventurous chapter as a postdoctoral fellow in the Division of Psychiatric Genomics at Icahn School of Medicine at Mount Sinai. Jiayi is looking forward to being further trained in genomics and psychiatric disorders to bring her interests of nutrition, genetics, and the brain together, and to eventually contribute to improving individual health and well-being.

谨以此书献给

- 生我爱我的父母和祖父母，感谢你们教会了我爱与被爱
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This work is dedicated to:

- My parents and grandparents, who love me unconditionally and fully support my every big decision. You have always been there rooting for me, accompanying me through my low points, and truly cheering for me for my every little accomplishment. I know from the bottom of my heart that even though I am living far away from you, I am not alone and you are my home.
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There are many people I would like to thank for the past 5 years of my PhD life. First and foremost, I wanted to thank my advisor, Pat Cassano, for her guidance, care and support since the very first meeting we had. Thinking back now, being her PhD student is probably one of the luckiest things that happened to me. Pat taught me so many things in research and beyond. As my dissertation advisor, Pat demonstrated a high standard for research integrity, rigor, and thoroughness. I learned from her to appreciate the value of perseverance when things did not turn out as expected. Besides that, the parts that I truly look up to are her life philosophy and the way she treats other people. Since living far away from home, Pat is the first person who encouraged me to take small breaks and get some exercise every day for a better body, mind and soul, and to celebrate every little milestone in life. She truly believed in me, supported me, and helped me being more confident in myself and in facing a future with uncertainty. Pat treats everyone around her with kindness, respect and genuineness. For the past 5 years, I have always felt motivated after meeting with Pat for my research work and she has never given away any frustrations or impatience. I am truly grateful for having Pat as my mentor and have enjoyed every moment of my life as a PhD student. All of this would not have been possible without the support from Pat.

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opportunities for me, and supported me to pursue my research passions. I am also very grateful to her willingness to squeeze time out of her busy schedule to provide feedback on my dissertation work before my defense. I felt very lucky to have my dissertation projects under the mentorship of both Dana and Pat, which was also a great opportunity for me to appreciate the scientific collaboration.

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CHAPTER 1

INTRODUCTION

Impact of Nutrition on Pulmonary Function

The association of nutritional status and dietary intake with pulmonary function and lung health has been studied over the past three decades (1). The proposed mechanisms for the effects of nutrients on lung outcomes relate to the balance of antioxidants and oxidants, as well as the balance of anti-inflammatory and inflammatory pathways. Chronic obstructive pulmonary disease (COPD) was the fourth leading cause of death in United States in 2016 (2) and a hallmark of COPD is airway and systemic inflammation (3). Cigarette smoking is a major causal factor for COPD, which leads to oxidative stress in the lungs (4), and smoking cessation is a key preventive approach to preserving pulmonary function and avoiding COPD. The studies of nutrition and pulmonary function investigate the hypothesis that diets rich in anti-inflammatory nutrients and/or antioxidants, such as vitamin D, omega-3 polyunsaturated fatty acids (n-3 PUFAs), and vitamin E, have protective effects against oxidative stress and inflammatory burden, with greater positive benefits of nutrition expected in cigarette smokers.

Effects of Vitamin D on Pulmonary Function

Biological Mechanism

The active form of vitamin D, 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D] acts as a steroid hormone through binding with the vitamin D receptor and the retinoid X receptor to regulate the expression of hundreds of genes that have a vitamin D response element (VDRE) in the promoter region (5). Genes with a VDRE are

associated with immune response and inflammatory response (6), suggesting an anti-inflammatory property of vitamin D, besides its well-known calcemic effect on bone health. Vitamin D plays an important role in both innate and adaptive immune systems because it can mediate the production of antimicrobial peptides, induce autophagy in human macrophages and promote the function and number of regulatory T cells (7). In addition, the vitamin D receptor and one vitamin D-related enzyme, CYP27B1, which produces the active form of vitamin D from its precursor, 25-hydroxyvitamin D [25(OH)D], were found to be expressed in airway epithelial cells, suggesting a local effect of vitamin D on lungs (8). Vitamin D was also shown to be involved in pathways related to inflammation in the airway (9, 10), development of pulmonary surface area (11), regulation of extracellular matrix homeostasis in lung tissue (12), and remodeling of the airway smooth muscle (7), lung volume (13), tracheal diameter, and alveolar counts (14). Animal studies showed that vitamin D supplementation improved pulmonary function and alveolar development in mice postnatally and stimulated alveolar regeneration in mice with COPD (14-16).

Epidemiological Evidence

25(OH)D, the commonly used biomarker for vitamin D, was positively associated with pulmonary function among adults (17-23), as well as among adolescents (23, 24) and COPD patients (25, 26) in cross-sectional studies. In some studies, the association differed by sex (27), smoking status (28), and BMI (29). In the few prospective cohort studies, a study with 20 years of follow-up reported that lower vitamin D was associated with faster FEV_1 decline in the general population (20), but a study with 6 years follow-up found no association (19). Other longitudinal studies showed the association only in smokers or in COPD patients (28, 30). A recent randomized controlled trial (RCT) reported an effect of vitamin D on pulmonary function limited to ever smokers (31); at the same time, this RCT and another pilot

RCT failed to show an effect of vitamin D in vitamin D-deficient or COPD patients (31, 32). No study thus far has investigated the consistency of the cross-sectional association of vitamin D and pulmonary function across ancestries or geographic regions, and the second chapter of my dissertation addressed this gap in knowledge.

Effects of N-3 PUFAs on Pulmonary Function

Biological Mechanism

N-3 PUFAs are important components of cell membranes, which affect membrane fluidity (33) and water permeation capability (34). Besides their function in cell membranes, n-3 PUFAs exert anti-inflammatory effects via several mechanisms, including *decreasing* the generation of pro-inflammatory eicosanoids (e.g., prostaglandin E2, leukotriene B4), inflammatory cytokines (e.g., tumor necrosis factor-α, interleukin-1β), reactive oxygen species, and *increasing* generation of DHA and EPA-derived anti-inflammatory resolvins (35, 36). Experimental studies showed that n-3 PUFAs, and/or the derived resolvins, increased pulmonary surface area (37), decreased collagen production and proliferation of lung fibroblasts, induced wound repair of alveolar type II cells (38, 39), and promoted alveolar fluid clearance (40). In animal intervention studies, n-3 PUFA supplementation led to better pulmonary function and less airway inflammation (e.g., decreased neutrophil and macrophage numbers) (39, 41).

Epidemiological Evidence

Several epidemiological studies investigated the association of omega-3 fatty acids with pulmonary function, mostly by measuring the dietary intake of n-3 PUFAs (42-47), with only a few studies measuring concentrations of n-3 PUFA biomarkers (48, 49). Overall, dietary n-3 PUFAs were positively associated with pulmonary function, regardless of how the dietary n-3 PUFA variable was parameterized (e.g., as

a categorical variable, such as daily supplemental n-3 PUFAs [yes/no] (44), or as a continuous variable, such as % of energy from n-3 PUFAs (45) or grams/day (46)). Among smokers, dietary DHA and DPA were positively associated with FEV_1, and dietary DPA was also associated with slower decline in FEV_1 (47). In contrast, a Dutch population-based study reported that a higher dietary intake of DHA was associated with lower FEV_1 (*n*=13,820) (42), and one small study (*n*=79) reported little to no association of dietary n-3 PUFAs with pulmonary function (43). To the best of our knowledge, only two studies investigated the association of n-3 PUFA biomarkers with pulmonary function. Serum phospholipid DHA was positively associated with predicted FEV_1% and FVC% in adult men (*n*=593) (49), and serum ALA was associated with predicted FEV_1% in children with borderline significance (p=0.057, *n*=526) (48).

In more clinically-focused studies, dietary n-3 PUFAs (50) and plasma n-3 PUFAs (51) were associated with COPD risk in smokers and with symptoms of chronic bronchitis in adolescents (52), but there was little to no association with risk of developing COPD in overall healthy populations (53). In omega-3 PUFA supplementation trials, a randomized controlled trial (RCT) in young male wrestlers (*n*=40) reported improved FEV_1 and FVC after 3 months of supplementation (54). Other n-3 supplementation trials, that either had a shorter study duration or smaller sample size, failed to show a beneficial effect of n-3 supplementation on pulmonary function (i.e., FEV_1 and FVC) (55-57).

Overall, definitive research on the role of omega-3 fatty acids in pulmonary function and lung health is lacking. In the third chapter of my dissertation, the relation of n-3 PUFA biomarkers with pulmonary function was examined in a large population-based sample (n > 16,000) that included European and African ancestry populations in several countries (U.S., Iceland and the Netherlands) with enough

variation in smoking to allow careful consideration of modification by smoking.

Effects of Vitamin E on Pulmonary Function

Biological Mechanism

Vitamin E is a fat-soluble vitamin with 8 different forms, including alpha (α), beta (β), gamma (γ), and delta (δ)-tocopherols and tocotrienols. In tissues and blood, α-tocopherol (α-TOH) is the primary form given the preferential post-absorptive elimination of other forms of vitamin E. In contrast, the primary form of vitamin E in the diet is γ-tocopherol, followed by δ-tocopherol, and then α-tocopherol. The major role of α-TOH in tissues is to serve as an antioxidant given its strong capacity to react with peroxyl radicals to break the chain reaction of lipid peroxidation and protect cell membranes from oxidant damage (58). Mechanistic studies showed that vitamin E is beneficial and preserves inflammatory response in aging. More specifically, vitamin E is proposed to attenuate or mitigate the age-associated T cell function defect through its effects on lymphocyte proliferation as well as by inhibiting prostaglandin E2 production via suppression of nitric oxide and superoxide (59).

Animal studies showed that a diet deficient in vitamin E led to a decrease in lung tidal volume in the presence of oxidative stress (60) compared to a vitamin E sufficient diet, and implementation of γ-tocotrienol improved pulmonary function, mitigated alveolar damage, and reduced neutrophil counts and other inflammatory biomarkers in mice with cigarette smoke-induced COPD (61). These animal studies support a true effect, and suggest the need for further research to identify the mechanism and efficacy of α-TOH supplementation on pulmonary function and COPD risk.

Epidemiological Evidence

Epidemiological studies investigated both dietary intake and biomarkers of

vitamin E in association with pulmonary function. Findings were inconsistent for dietary vitamin E intake; some studies reported positive associations with pulmonary function (62-66), some reported null findings (67-70), and several studies reported positive associations that varied by smoking status (43), country (71), and/or sex (72). Among the studies that measured vitamin E biomarkers as the exposure, most focused on alpha-tocopherol since it is the primary form of vitamin E transported in blood circulation. About half of the studies reported positive associations with pulmonary function (69, 73-76) while the other half reported little to no association (65, 77-81). Among these studies, those reporting positive associations had sample sizes greater than 1,000; in comparison, the studies reporting little to no association had sample sizes less than 1,000. The studies investigating other vitamin E forms (e.g., gamma-tocopherol) reported an inverse association of serum gamma-tocopherol level with pulmonary function (65, 76). Only two prospective studies investigated the association of vitamin E with longitudinal pulmonary function decline; one measured dietary intake (63) and the other measured serum level (82). Both reported little to no association overall, but one of these studies reported that lower baseline serum vitamin E level was associated with accelerated pulmonary function decline only in heavy smokers (82).

In a study of a clinical outcome, dietary vitamin E intake was associated with a lower risk of COPD (66), and there was no evidence that the effect differed by smoking status. To the best of our knowledge, very few randomized controlled trials (RCTs) investigated the effect of vitamin E supplementation on pulmonary function. A recent published RCT conducted in our research group reported no effect of vitamin E supplementation on pulmonary function decline compared to the placebo group ($p = 0.1866$), and no evidence for an interaction of vitamin E supplementation with smoking status on decline (83). In light of the significant degree of inter-individual

variation in plasma vitamin E status in response to vitamin E supplementation (84), the RCT findings in the intent-to-treat analysis may understate the effect of the intervention. It is feasible that plasma change in vitamin E level varies significantly across participants given an identical dose of vitamin E supplementation, and thus the intervention effect is expected to vary. It is well-known that for nutrition interventions the baseline level of a nutrient may be a primary driver of response, and the beneficial effects of the intervention may be limited to subgroups (85). My third dissertation project sought to fill the gap by investigating the relation of individual change in plasma vitamin E (both alpha- and gamma-tocopherol) with rate of decline in pulmonary function after supplementation, as well as to identify genetic variants associated with plasma response to vitamin E supplementation.

The Genetics of Pulmonary Function

Pulmonary function develops from birth to late adolescence, reaches its peak at early adulthood, stays stable for a few years and then starts to decline slowly but steadily over the rest of adult life (86). An accelerated pulmonary function decline, commonly seen in smokers, is a risk factor for COPD development (87). To diagnose COPD, a pulmonary function test is conducted to measure forced expiratory volume in the first second (FEV_1, a measure of airway obstruction), forced vital capacity (FVC, a measure of lung volume), and to calculate the ratio of FEV_1/FVC. A ratio of FEV_1/FVC less than 70% and a low percentage predicted FEV_1 (predicted FEV_1%, the ratio of individual FEV_1 over the predicted FEV_1 for a population of the same height, age, gender and race) are indicative of the degree of airway obstruction and the severity of COPD (88).

Genetic heritability studies provide evidence that both genetic and environmental factors are important determinants of pulmonary function and the

average heritability for cross-sectional pulmonary function was ~50% (89-91) while the average heritability for longitudinal pulmonary function was ~10% overall and ~25% in smokers (92). For COPD, the genetic heritability was estimated at ~38% (93). To date, genome-wide association studies (GWAS) identified more than 270 genetic loci associated with cross-sectional pulmonary function at genome-wide significance ($P < 5 \times 10^{-8}$), primarily in populations of European ancestry (94-103). Among these loci, some were associated with pulmonary function across multiple ancestry groups (e.g., African, Asian, and Hispanic) (99, 102), while some were ancestry-specific given allelic differences in different populations. In addition, 95 of these loci were extended to test their associations with COPD, and about 32% of genetic variants were associated with COPD risk (101). Recent functional follow-up studies of candidate genetic loci from previous GWAS revealed novel mechanisms that may underlie the role these genes (e.g., *AGER, EGLN2, FAM13A, HHIP, HTR4*) play in pulmonary function and COPD (104-108). In contrast, to the best of our knowledge, there have not been any reports of replicated genome-wide significant variants for the longitudinal phenotype of pulmonary function decline (109-111).

Gene-by-Nutrient Interaction on Pulmonary Function

Diet is one important lifestyle factor that is hypothesized to play a role in pulmonary function. The heritability of cross-sectional pulmonary function is ~50% (89-91), suggesting the environmental factors and gene-by-environment interactions to explain the other 50% of variability in pulmonary function.

There is a strong biological premise for gene-by-environment, or more specifically gene-by-nutrient interactions. Based on individual genetic variation, the metabolic efficiency of diet and nutrients is expected to vary from person to person (e.g., lactose intolerance (112), gluten intolerance (113), and alcohol flush (114)).

Furthermore, absorption of nutrients leads to many downstream effects on biological pathways including effects on epigenetic modification and/or gene expression regulation (115). For example, in lung tissues, vitamin D regulates the expression of genes related to inflammatory responses through binding with the VDRE in the gene promoter region (116), supporting a biological mechanism for the proposed effect of vitamin D on lungs (117). The anti-inflammatory omega-3 fatty acids (e.g., DHA, DPA) are proposed to down-regulate the expression of pro-inflammatory genes in lungs by altering transcription factor activation (35). Given the antioxidant role of vitamin E to protect membrane lipids against peroxidation and to maintain cell membrane stability, vitamin E is proposed to indirectly modulate the lipid-derived mediators that, in turn, affect gene expressions involved in tissue inflammation (e.g., lungs) (118). The above considerations outline reasonable biological mechanisms that lead to hypotheses about gene-by-nutrients interaction effects on pulmonary function that are investigated in this dissertation research.

Dissertation Overview

To examine the potential benefits of dietary modification on lung health, my dissertation studied the association of nutrient biomarkers that have anti-inflammatory and antioxidant functions (i.e., vitamin D, omega-3 fatty acids, and vitamin E) with pulmonary function on a population level across different ancestry groups (part I of **Figure 1**). In addition, the interaction of genetic variants with nutrients on pulmonary function was investigated (part II of **Figure 1**). We worked on a genome-wide scale to identify population subgroups that are susceptible to impaired pulmonary function and to discover mechanisms through which dietary modification plays a beneficial role in protecting pulmonary function. This research also examined change in plasma vitamin E concentration in response to an intervention in relation to the longitudinal

pulmonary function phenotype, and identified genetic factors contributing to the differences in response to vitamin E supplementation across individuals (part III of **Figure 1**).

The first manuscript investigated the association of serum vitamin D biomarker, 25(OH)D, with pulmonary function in European and African ancestry populations across multiple cohorts. We leveraged data from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium and conducted a meta-analysis. In addition, given the role of cigarette smoking in contributing to high oxidative stress in the lungs, the interaction of 25(OH)D with smoking status on pulmonary function was investigated. Heterogeneity across the cohorts was examined to evaluate the consistency and generalizability of the findings.

The second manuscript identified a gene-by-nutrient interaction; the negative effect of the genetic variant on pulmonary function was attenuated in persons with higher plasma omega-3 fatty acid status (i.e., DHA). For this analysis, a meta-analysis was performed in the two ancestry groups across multiple cohorts to identify specific n-3 PUFAs associated with pulmonary function. Secondly, we conducted a genome-wide interaction analysis of these n-3 PUFAs (i.e., DHA and DPA) to identify the genetic susceptibility to pulmonary function that was modified by n-3 PUFA concentrations in the blood.

The third manuscript investigated the association of change in plasma vitamin E concentration (alpha- and gamma-tocopherol) with change in pulmonary function after 3 years of vitamin E supplementation. Genome-wide association analyses and look-up of significant loci reported in the previous GWAS (119) led to replication of a genetic variant that was related to plasma response to vitamin E supplementation. The findings from this work are expected to contribute to the knowledge base of vitamin E supplementation efficacy on pulmonary function in different populations.

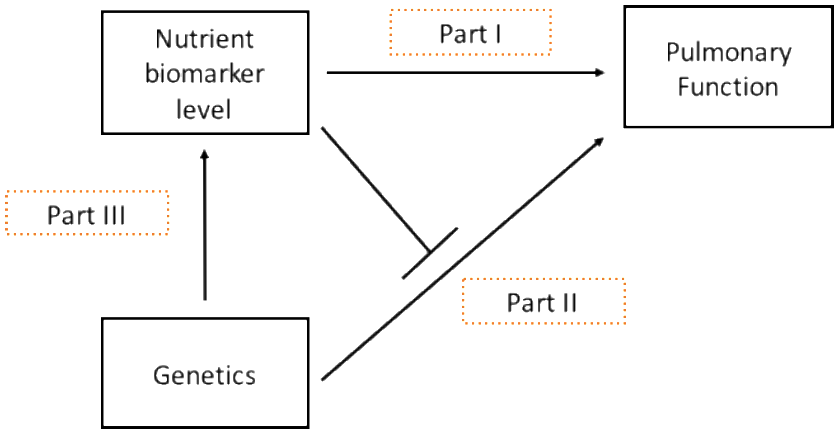

Figure 1. Conceptual framework of the dissertation

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CHAPTER 2

META-ANALYSIS ACROSS COHORTS FOR HEART AND AGING RESEARCH IN GENOMIC EPIDEMIOLOGY (CHARGE) CONSORTIUM PROVIDES EVIDENCE FOR AN ASSOCIATION OF SERUM VITAMIN D WITH PULMONARY FUNCTION

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Meta-analysis across Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium provides evidence for an association of serum vitamin D with pulmonary function

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Abbreviations: 1,25-$(OH)_2$D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; AA, African ancestry; AGES, Age, Gene, Environment, Susceptibility Study – Reykjavik, Iceland; ARIC, Atherosclerosis Risk in Communities Study; CARDIA, Coronary Artery Risk Development in Young Adults Study; CHARGE, Cohorts for Heart and Aging Research in Genomic Epidemiology; CHS, Cardiovascular Health Study; COPD, chronic obstructive pulmonary disease; EA, European ancestry; FEV_1, forced expiratory volume in the 1st second; FHS (Offspring), Framingham Heart Study –Offspring Cohort; FHS (Gen3), Framingham Heart Study – Generation 3 Cohort; FVC, forced vital capacity; HABC, Health, Aging, and Body Composition Study; MESA, Multi-Ethnic Study of Atherosclerosis; PFT, pulmonary function test; RIA, radioimmunoassay.

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Abstract

The role that vitamin D plays in pulmonary function remains uncertain. Epidemiological studies reported mixed findings for serum 25-hydroxyvitamin D (25(OH)D)–pulmonary function association. We conducted the largest cross-sectional meta-analysis of the 25(OH)D–pulmonary function association to date, based on nine European ancestry (EA) cohorts (n 22 838) and five African ancestry (AA) cohorts (n 4290) in the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium. Data were analysed using linear models by cohort and ancestry. Effect modification by smoking status (current/former/never) was tested. Results were combined using fixed-effects meta-analysis. Mean serum 25(OH)D was 68 (SD 29) nmol/l for EA and 49 (SD 21) nmol/l for AA. For each 1 nmol/l higher 25(OH)D, forced expiratory volume in the 1st second (FEV_1) was higher by 1·1 ml in EA (95 % CI 0·9, 1·3; $P < 0·0001$) and 1·8 ml (95 % CI 1·1, 2·5; $P < 0·0001$) in AA ($P_{race\ difference} = 0·06$), and forced vital capacity (FVC) was higher by 1·3 ml in EA (95 % CI 1·0, 1·6; $P < 0·0001$) and 1·5 ml (95 % CI 0·8, 2·3; $P = 0·0001$) in AA ($P_{race\ difference} = 0·56$). Among EA, the 25(OH)D–FVC association was stronger in smokers: per 1 nmol/l higher 25(OH)D, FVC was higher by 1·7 ml (95 % CI 1·1, 2·3) for current smokers and 1·7 ml (95 % CI 1·2, 2·1) for former smokers, compared with 0·8 ml (95 % CI 0·4, 1·2) for never smokers. In summary, the 25(OH)D associations with FEV_1 and FVC were positive in both ancestries. In EA, a stronger association was observed for smokers compared with never smokers, which supports the importance of vitamin D in vulnerable populations.

Key words: Vitamin D: Forced expiratory volume: Vital capacity: Respiratory function tests: Smoking: Whites: African Americans

Chronic obstructive pulmonary disease (COPD), the third leading cause of mortality in the USA[1] and among the top ten leading causes of total years of life lost in the world[2], is characterised by progressive airway obstruction. Pulmonary function tests (PFT), as performed by spirometry, are used to quantify pulmonary function parameters including forced expiratory volume in the 1st second (FEV_1) and forced vital capacity (FVC). Pulmonary function increases throughout childhood, plateaus in the 20s, and thereafter adults experience an age-related decline[3]. The majority of COPD cases (85 %) are related to smoking[4], which alters the trajectory in pulmonary function, by hindering growth, reducing peak function and accelerating age-related decline[5].

Vitamin D is proposed to have protective effects in the lungs via gene regulation[6]. *In vitro* studies found that 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D), the active vitamin D metabolite, induced antimicrobial peptides for host defence in the lung and modulated airway remodelling[7]. In humans, 25-hydroxyvitamin D (25(OH)D) is the major vitamin D metabolite in serum, most of which forms a complex with vitamin D binding protein (DBP) (approximately 85–90 % is DBP-bound)[8], and then is metabolised to 1,25-$(OH)_2$D, the active steroid hormone form[8,9]. Total 25(OH)D is the commonly used biomarker of vitamin D status, and it is preferred to other vitamin D metabolites, such as non-DBP-bound 25(OH)D and 1,25-$(OH)_2$D, given that it is a comprehensive indicator for vitamin D stores, has a longer half-life (approximately 3 weeks) and is less affected by Ca[10,11]. On average, African ancestry (AA) populations have lower serum 25(OH)D concentrations,

due to multiple factors including genetics and skin pigmentation[7], but evidence exists that AA populations have higher 1,25-$(OH)_2$D levels and greater bone mineral density compared with European ancestry (EA) populations[12].

Previous observational cross-sectional studies of the vitamin D–pulmonary function association in the general population reported mixed findings. Most of these studies reported a positive association between 25(OH)D and pulmonary function[13–19], although some reported a null or inverse association[20–22], and two others reported a positive association under certain conditions, such as only in male current smokers[23] or only in overweight and obese males[24]. The largest previous cross-sectional study, which included two Danish cohorts (total 18 507), reported positive associations of 25(OH)D with pulmonary function[16]. Only one prior cross-sectional study investigated serum 25(OH)D and pulmonary function in an ancestry group other than European, and it confirmed similar positive associations in the 3957 AA participants studied[13].

The current study investigated the hypothesis that serum 25(OH)D level is positively associated with pulmonary function. We leveraged the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium to include population-based data on serum 25(OH)D and pulmonary function in a harmonised analysis. In addition, we compared the association of serum 25(OH)D and pulmonary function across EA and AA groups and investigated effect modification by cigarette smoking.

Methods

Cohorts and participants

Nine prospective cohorts in the CHARGE Consortium were included (Table 1). All cohorts had EA participants, and five of the cohorts had AA participants. Only one cohort (Multi-Ethnic Study of Atherosclerosis (MESA)) has participants with other ancestries, and these other ancestries were not included in this study. Among the nine cohorts, the Framingham Heart Study (FHS) had two sub-cohorts analysed separately: the Offspring and the Third-Generation (Gen3) cohorts. Our analysis pipeline harmonised the outcome and exposure definitions, the units on all variables and the statistical modelling. The same exclusion criteria were applied to each cohort: missing PFT, unacceptable PFT using the American Thoracic Society and European Respiratory Society criteria for acceptability, missing serum 25(OH)D, serum 25(OH)D >374·4 nmol/l (or 150 ng/ml, leading to removal of a single outlier)[25] or missing on other covariates (online Supplementary Table S1).

Outcome and exposure assessment

Pre-bronchodilator pulmonary function outcomes (FEV_1, FVC and FEV_1/FVC), which have similar accuracy as post-bronchodilator measures for long-term outcomes[26], were measured in each cohort using standardised methods defined by the American Thoracic Society/European Respiratory Society criteria (online Supplementary Table S2). The methods used to measure 25(OH)D varied by cohort (online Supplementary Table S2). Three cohorts, including MESA, the Atherosclerosis Risk In Communities (ARIC) study, and the Cardiovascular Health Study (CHS), used the current reference method, liquid chromatography in tandem with mass spectrometry (LC-MS/MS); three cohorts, including FHS, the Coronary Artery Risk Development in Young Adults (CARDIA) study, and the Health, Aging, and Body Composition (HABC) study, used radioimmunoassay (RIA); one cohort, the Age, Gene, Environment, Susceptibility Study – Reykjavik, Iceland (AGES), used chemiluminescence immunoassay (CLIA); and one cohort (the Rotterdam Study (RS)) used electro-CLIA. Only MESA calibrated the serum 25(OH)D measurement against the standard reference material 972[27], which reflects the calendar time of the measurements in the cohorts, most of which occurred before the availability of the standard reference material (online Supplementary Table S3). Measurements of the outcome and exposure variables were planned for either the full cohort (ARIC, CHS, FHS, HABC and RS) or a subset of the cohort if the outcome or the exposure was only measured in an ancillary study (AGES, CARDIA and MESA)[28–31] (online Supplementary Table S1). Continuous variables were used for serum 25(OH)D and pulmonary function to capture the association of 25(OH)D on PFT across the broad distribution of ranges in the cohorts.

As shown in Table 1, among nine cohorts, four (AGES, CHS, FHS-Offspring and FHS-Gen3) had a mean time difference of <1 year in the PFT measurements and the preceding 25(OH)D measurement, and the greatest mean time difference between 25(OH)D and PFT measurement was <5 years (MESA). Participants in ARIC and HABC had blood drawn for serum 25(OH)D after their PFT measure, but within 3 years.

Other covariates, including smoking status, pack-years (number of packs of cigarettes smoked per d times the number of years smoked), height, weight and age, were measured concurrently with pulmonary function, except for CHS, which assessed covariates concurrent with the serum 25(OH)D measure, but within 1 year of the PFT measurement (online Supplementary Table S3). All data collection and analysis was approved by the Institutional Review Board at each cohort's respective institution. Spirometry measures are available on the database of Genotypes and Phenotypes via accession numbers as follows: ARIC (phs000280), CARDIA (phs000285), CHS (phs000287), FHS (phs000007) and MESA (phs000209). Serum vitamin D measures are also available at the same accession numbers for CHS, FHS and MESA.

Statistical analysis in individual cohorts

All analyses were first conducted independently in each cohort, stratified by ancestry, given the lower mean serum 25(OH)D level in AA participants[7]. For FEV_1 and FEV_1/FVC, models were adjusted for smoking status, pack-years, height, height squared, age, age squared, sex, season of blood draw and study centre (if applicable); for FVC, the model was further adjusted for weight. Residual outliers, identified using the studentised residuals of the linear models (online Supplementary methods for more details), were excluded from all models (about 0·3 % of the total sample size). The model was extended to test the

interaction between 25(OH)D and smoking status (never (reference group), former and current smokers).

Meta-analysis

We tested the association of serum 25(OH)D on each PFT outcome among individuals in each ancestry group and each cohort, separately, and then combined the effect estimates (also referred to as two-stage meta-analysis[32]), using inverse variance weighting and assuming fixed-effects, with heterogeneity assessed via the I^2 statistic[33]. Random-effects meta-analysis was performed as a sensitivity analysis if there was potential heterogeneity ($I^2 > 30\%$). The comparison of meta-analysed coefficients of the 25(OH)D–PFT associations for the two ancestry groups was conducted using a Z test[34]. Meta-analysis of the interaction terms of 25(OH)D with smoking status was also performed (online Supplementary methods for more details, online Supplementary Tables S4 and S5 for cohort-specific findings and online Supplementary Table S6 for meta-analysed results).

Meta-regression was conducted to explore the potential causes of moderate heterogeneity in the meta-analysis of 25(OH)D on FEV_1 and FVC in the EA cohorts. Modifiers were tested individually in the meta-regression models to investigate heterogeneity; modifiers included factors that could vary between cohorts, such as proportion of ever, current and former smokers, mean 25(OH)D level, assay method for serum 25(OH)D, time between 25(OH)D and PFT measures, and mean age of participants in each cohort. The two-sided type I error was examined at 0·05 for all analyses. Meta-analysis and meta-regression were conducted using the metafor package (version 1.9-8) in R (version 3.2.3.; R Foundation for Statistical Computing).

Regression coefficients (β) with their standard errors calculated within each cohort per 1 nmol/l 25(OH)D are presented in the figures. In addition, to put the magnitude of the 25(OH)D–PFT associations in terms relevant to public health, the meta-analysed regression coefficients were multiplied by 10 nmol/l 25(OH)D, which is about half of the standard deviation of the 25(OH)D distribution.

Results

We studied 22 838 EA and 4290 AA participants. EA participants had higher FEV_1, FVC and serum 25(OH)D than AA participants in each cohort, whereas FEV_1/FVC was similar across ancestry groups (Table 1 and online Supplementary Fig. S1). CARDIA and FHS-Gen3 were younger than the seven other cohorts, with consequently lower pack-years smoked in ever smokers. Across all cohorts, among EA participants, 17 % were current smokers and 40 % were former smokers; among AA participants, 22 % were current smokers and 30 % were former smokers. The mean serum 25(OH)D level was highest among never smokers (70 (SD 30) nmol/l), followed by former smokers (67 (SD 29) nmol/l) and current smokers (64 (29) nmol/l) in EAs, whereas the trend was less obvious in AA (49 (SD 21) nmol/l in current smokers, 50 (SD 21) nmol/l in former smokers and 48 (SD 21) nmol/l in never smokers). The mean of serum 25(OH)D for EA participants across nine cohorts was 68 (SD 29) nmol/l and for AA participants across five cohorts the mean was 49 (SD 21) nmol/l.

Fixed-effects meta-analysis (Fig. 1) revealed a consistently positive association of serum 25(OH)D with the PFT outcomes, FEV_1 and FVC, in both ancestry groups. To put these findings into context, a 10 nmol/l (approximately 0·5 SD) higher 25(OH)D was associated with 11·1 ml higher FEV_1 in EA ($P < 0{\cdot}0001$) and 17·9 ml higher FEV_1 in AA ($P < 0{\cdot}0001$). Similarly, for a 10 nmol/l higher 25(OH)D, FVC was higher by 12·9 ml in EA ($P < 0{\cdot}0001$) and by 15·4 ml in AA ($P = 0{\cdot}0001$). The magnitudes of the 25(OH)D–PFT associations did not differ significantly between the two ancestry groups ($P = 0{\cdot}06$ and $P = 0{\cdot}56$ for FEV_1 and FVC, respectively). The association of serum 25(OH)D with FEV_1/FVC reached statistical significance only in EA ($P = 0{\cdot}0013$), and the magnitude was negligible; a 10 nmol/l higher 25(OH)D was associated with a ratio being lower by 0·055 % (online Supplementary Table S7 and Supplementary Fig. S2 for ancestry- and cohort-specific findings).

In the main-effect meta-analysis of serum 25(OH)D on pulmonary function, EA cohorts had low to moderate heterogeneity, whereas AA cohorts had low heterogeneity (Fig. 1, online Supplementary Fig. S2). We did a sensitivity analysis using random-effects meta-analysis among EA cohorts for the FEV_1 and FVC outcomes, and no substantial change was found in the meta-analysed effect estimates and corresponding SE (coefficient of 1 nmol/l 25(OH)D on the FEV_1 outcome was 1·11 (SE 0·12) ml in the fixed-effects model and 1·21 (SE 0·19) ml in the random-effects model; coefficient on the FVC outcome was 1·29 (SE 0·14) ml in the fixed-effects model and 1·31 (SE 0·20) ml in the random-effects model). Meta-regression was also performed in the EA cohorts and we found that among these cohorts, cohorts with lower mean 25(OH)D concentration had stronger 25(OH)D–PFT associations (Fig. 2). The proportion of ever smokers and of former smokers had significant linear associations with the 25(OH)D–PFT coefficients (online Supplementary Fig. S3), and these two variables were both highly correlated with mean 25(OH)D levels (Pearson's $r < -0{\cdot}75$ for all pairwise correlations). The 25(OH)D–PFT association in EA cohorts varied by 25(OH)D assay method (meta-regression $P = 0{\cdot}0059$); the association was attenuated in cohorts using RIA compared with cohorts using liquid chromatography in tandem with MS (pairwise $P < 0{\cdot}005$, online Supplementary Fig. S4). Mean age of each cohort was a significant positive modifier of the 25(OH)D–FEV_1 association, while time difference between 25(OH)D and spirometry measures did not affect the 25(OH)D–PFT association (online Supplementary Fig. S3).

To examine the potential impact of family relatedness between the FHS-Gen3 and the FHS-Offspring cohorts on the meta-analysis, sensitivity analysis confirmed that the findings were unchanged when either cohort was excluded (results not shown). In addition, the meta-analysis findings were not sensitive to exclusion of residual outliers.

In the EA cohorts, 25(OH)D had a greater positive association with FVC in current smokers than in never smokers ($\beta_{\text{current} \times 25(OH)D} =$ 7·5 ml for 10 nmol/l increment of 25(OH)D, $P = 0{\cdot}047$). Similarly, 25(OH)D had a greater positive association with FVC

Table 1. Cross-sectional participant characteristics of each cohort in the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium (*n* 27 128)*
(Mean values and standard deviations)

	ARIC		CARDIA		CHS†		HABC‡		MESA		AGES		FHS (Offspring)		FHS (Gen3)		RS	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
European ancestry cohorts																		
Number of participants	8327		172		1297		1411		1113		1685		1639		3610		3584	
Males (%)	46·0		58·7		30·2		53·3		49·2		40·8		48·1		47·3		44·6	
Current smoker (%)	23·4		11·6		9·4		6·5		8·4		9·8		14·3		15·3		16·0	
Former smoker (%)	34·9		16·3		44·9		49·8		47·2		42·4		50·5		28·0		52·9	
Pack-years§	28·0	20·9	6·2	7·2	28·1	25·3	36·4	32·0	30·1	29·6	24·6	21·9	26·5	22·8	12·4	13·4‖	22·9	21·6
Age (years)	54·2	5·7	34·8	3·1	73·7	4·4	73·7	2·8	66·3	9·9	76·2	5·6	59·4	9·3	40·2	8·7	64·4	9·7
Height (m)	1·69	0·09	1·73	0·09	1·63	0·09	1·67	0·09	1·69	0·10	1·67	0·09	1·68	0·09	1·71	0·09	1·69	0·09
Weight (kg)¶	76·8	16·2	76·9	17·0	70·6	14·2	74·5	14·5	79·7	17·3	75·4	14·7	79·4	17·2	78·6	18·4	79·5	14·6
FEV_1 (ml)	2946	767	3881	743	2010	611	2324	649	2556	768	2142	670	2724	757	3592	787	2848	866
FVC (ml)	3987	973	4967	999	2881	829	3118	810	3492	995	2877	837	3711	950	4621	999	3692	1063
FEV_1/FVC	0·739	0·077	0·785	0·060	0·700	0·095	0·745	0·078	0·734	0·087	0·744	0·087	0·733	0·078	0·779	0·063	0·771	0·082
Serum 25(OH)D (nmol/l)**	64·7	21·8	95·0	35·3	68·0	27·9	72·2	25·6	75·6	28·2	52·4	23·5	49·2	18·9	92·8	36·0	61·0	27·4
Never smoker	64·3	21·0	95·4	34·4	67·1	25·1	73·7	25·9	76·5	27·7	54·1	22·8	49·6	18·6	93·2	35·4††	59·7	25·9
Former smoker	67·1	21·5	94·5	43·0	69·4	29·4	71·7	24·8	76·2	28·5	52·3	24·1	49·8	18·6	93·5	37·0	62·3	27·7
Current smoker	61·8	23·1	92·7	29·5	65·4	33·2	65·0	28·1	66·9	28·2	44·5	22·7	45·9	20·6	89·9	36·3	59·5	29·4
Method of 25(OH)D measurement	LC-MS/MS		RIA		LC-MS/MS		RIA		LC-MS/MS		CLIA		RIA		RIA		Electro-CLIA	
Time from 25(OH)D to PFT (d)‡‡	−1073	67	1122	89	363	29	−382	39	1765	112	1	5	133	377	2	61	846	808
Season of 25(OH)D measurement (%)§§																		
Spring	31·2		8·1		20·5		30·5		29·0		22·4		29·2		26·8		29·6	
Summer	26·1		36·1		30·1		18·1		22·2		12·4		11·0		29·6		18·9	
Autumn	23·3		34·3		29·6		22·8		24·9		33·8		29·1		24·1		30·0	
Winter	19·5		21·5		19·8		28·6		23·9		31·4		30·7		19·4		21·5	
African ancestry cohort																		
Number of participants	2339		157		168		863		763									
Males (%)	35·3		51·6		25·6		44·5		47·4									
Current smoker (%)	27·5		26·1		10·7		15·8		15·7									
Former smoker (%)	23·9		9·6		42·9		39·3		38·3									
Pack-years§	21·4	20·7	5·3	4·6	21·9	18·3	29·4	23·4	23·6	21·8								
Age (years)	53·3	5·7	33·9	3·2	71·9	4·5	73·4	2·9	65·6	9·7								
Height (m)	1·68	0·09	1·71	0·10	1·63	0·08	1·66	0·09	1·68	0·10								
Weight (kg)¶	83·5	17·1	82·2	16·9	75·7	13·3	78·2	15·1	84·3	16·8								
FEV_1 (ml)	2495	638	3237	709	1801	508	1958	566	2200	667								
FVC (ml)	3255	806	4077	920	2507	706	2594	712	2933	869								

Table 1. *Continued*

	ARIC		CARDIA		CHS†		HABC‡		MESA		AGES		FHS (Offspring)		FHS (Gen3)		RS	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
FEV_1/FVC	0·768	0·077	0·799	0·070	0·723	0·076	0·757	0·090	0·755	0·093								
Serum 25(OH)D (nmol/l)**	47·4	17·5	69·4	31·2	44·6	21·1	51·8	22·4	47·9	22·3								
Never smoker	46·8	16·7	71·3	30·1	43·7	19·2	51·8	22·7	49·1	22·3								
Former smoker	48·5	18·0	69·2	35·6	47·2	24·2	52·3	21·8	49·3	22·6								
Current smoker	47·5	18·4	64·8	32·4	38·3	14·9	50·4	23·2	40·9	20·0								
Method of 25(OH)D measurement	LC-MS/MS		RIA		LC-MS/MS		RIA		LC-MS/MS									
Time from 25(OH)D to PFT (d)‡‡	−1054	114	1101	104	350	26	−390	53	1719	115								
Season of 25(OH)D measurement (%)§§																		
Spring	30·0		10·2		58·9		35·6		34·6									
Summer	30·7		56·0		7·1		16·2		23·5									
Autumn	20·7		23·6		8·9		24·9		19·7									
Winter	18·6		10·2		25·0		23·3		22·3									

ARIC, Atherosclerosis Risk in Communities Study; CARDIA, Coronary Artery Risk Development in Young Adults Study; CHS, Cardiovascular Health Study; HABC, Health, Aging, and Body Composition Study; MESA, Multi-Ethnic Study of Atherosclerosis; AGES, Age, Gene, Environment, Susceptibility Study – Reykjavik, Iceland; FHS (Offspring), Framingham Heart Study – Offspring Cohort; FHS (Gen3), Framingham Heart Study – Generation 3 Cohort; RS, Rotterdam Study (Netherlands); FEV_1, forced expiratory volume in the 1st second; FVC, forced vital capacity; 25(OH)D, 25-hydroxyvitamin D; LC-MS/MS, liquid chromatography in tandem with MS; CLIA, chemiluminescence immunoassay; RIA, radioimmunoassay.

* AGES, RS and FHS only have participants of European ancestry; *n* 22 838 for EA, *n* 4290 for AA, total *n* 27 128.

† The number of participants used to compute descriptive statistics in CHS excluded those who had residual outliers based on the preliminary models (*n* 8 for EA and *n* 6 for AA); whereas other cohorts used the number of participants before applying residual exclusion for the descriptive statistics.

‡ Numbers vary slightly for different outcomes in HABC (for the FVC outcome, *n* 1385 for EA and *n* 821 for AA; for the ratio outcome, *n* 1382 for EAs and *n* 817 for AAs). The numbers of participants for the FEV_1 outcome are used. However, the descriptive statistics is similar across different outcomes.

§ Pack-years is calculated only among current and former smokers in each cohort.

‖ We used 1554 ever smokers here, instead of a total of 1561 ever smokers in the Gen3 cohort, because the pack-years of seven ever smokers were so small that they were coded as 0. Therefore, these seven ever smokers do not contribute to the pack-years descriptive statistics here.

¶ The number of participants who have weight data is slightly different from the total number of participants in each cohort. However, the descriptive statistics of weight stays similar.

** Mean (SD) of serum 25(OH)D level for all the participants in each cohort, and mean (SD) of 25(OH)D level in participants with each smoking status are shown here, stratified by ancestry.

†† We used 2046 never smokers, rather than a total of 2049 never smokers in the Gen3 cohort, to compute the 25(OH)D level in never smokers.

‡‡ The time difference is the interval between the time when pulmonary function was measured and the time when serum vitamin D was measured. The difference is positive, if the serum vitamin D was measured before the pulmonary function test; whereas the value is negative, if the serum vitamin D was measured after the pulmonary function test.

§§ The proportion of participants in each season when their serum was measured was rounded (thus rounding errors mean sums may not be exactly 100 %).

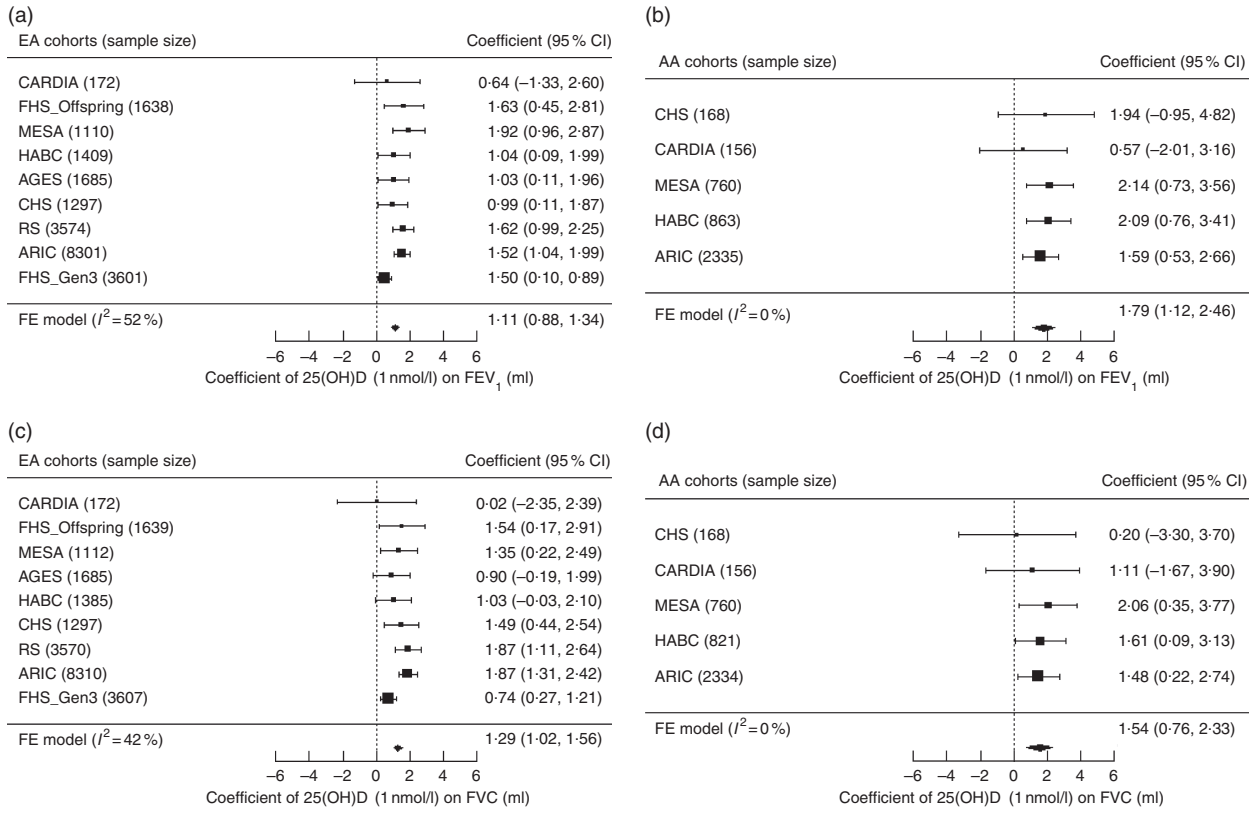

Fig. 1. Forest plots of the meta-analysis of serum 25-hydroxyvitamin D (25(OH)D) on forced expiratory volume in the 1st second (FEV_1) and forced vital capacity (FVC) across cohorts in the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium, stratified by participant ancestry. Associations are presented for serum 25(OH)D on (a) FEV_1 in European ancestry cohorts (*n* 22 787). (b) FEV_1 in African ancestry cohorts (*n* 4282). (c) FVC in European ancestry cohorts (*n* 22 777). (d) FVC in African ancestry cohorts (*n* 4239). β (unit: ml) denotes the coefficient from the fixed-effects meta-analysis for serum 25(OH)D on the pulmonary function outcome per 1 nmol/l increment of 25(OH)D, with its 95 % CI. Cohorts findings were ordered from the least to the most precise, and heterogeneity is presented (I^2). EA, European ancestry; AA, African ancestry; CARDIA, Coronary Artery Risk Development in Young Adults Study; FHS (Offspring), Framingham Heart Study – Offspring Cohort; AGES, Age, Gene, Environment, Susceptibility Study – Reykjavik, Iceland; CHS, Cardiovascular Health Study; RS, Rotterdam Study (Netherlands); ARIC, Atherosclerosis Risk in Communities Study; FHS (Gen3), Framingham Heart Study – Generation 3 Cohort; FE, fixed-effects; HABC, Health, Aging, and Body Composition Study; MESA, Multi-Ethnic Study of Atherosclerosis.

in former smokers than in never smokers ($\beta_{\text{former} \times 25(OH)D} = 7{\cdot}9$ ml for 10 nmol/l increment of 25(OH)D, $P = 0{\cdot}0065$) (Fig. 3). For the FEV_1 outcome in the EA cohorts, the interaction coefficients for 25 (OH)D and smoking status had the same positive direction as the coefficients for FVC, but were not statistically significant for either current ($P = 0{\cdot}14$) or former smokers ($P = 0{\cdot}14$). No statistical evidence of interaction of 25(OH)D and cigarette smoking was found in the AA cohorts for either outcome. To put the interaction finding into context, a 10 nmol/L higher serum 25(OH)D was associated with a 17·3 ml higher FVC in current smokers and a 16·6 ml higher FVC in former smokers, which was more than double the association magnitude in never smokers ($\beta = 7{\cdot}8$ ml). A similar trend was found for the FEV_1 outcome in the EA cohorts. For 10 nmol/l higher serum 25(OH)D, FEV_1 was higher by 14·0 ml in current smokers, 12·0 ml in former smokers and 8·0 ml in never smokers (Fig. 4).

Discussion

This study investigated the association of serum 25(OH)D with pulmonary function using multiple cohorts of different ancestries. We found a consistently positive association of serum 25 (OH)D with FEV_1 and FVC across both EA and AA groups. In addition, in the EA group, a significantly stronger association was observed for current and former smokers, compared with never smokers.

A previous cross-sectional study in a EA population (two Copenhagen cohorts: *n* 10 116 and *n* 8391, respectively) similarly reported positive associations of 25(OH)D with FEV_1 percentage predicted and FVC percentage predicted, but not with FEV_1/FVC[16]. The magnitude of the association was about four times greater in the Copenhagen study, which may be due to the difference in the mean serum 25(OH)D (Danish median approximately 42 nmol/l *v.* CHARGE median of approximately 65 nmol/l) given our finding that the 25(OH)D–PFT association was stronger in cohorts with lower serum 25(OH)D. Our finding for the serum 25(OH)D–FEV_1 association was similar in magnitude to the association reported in a British cohort of 6789 participants with an average age of 45 years[17], but weaker than a previous report from the FHS cohort[15]. Given that the rate of decline in FEV_1 at age 45 years is increased by approximately 15 ml/year in current smokers[35], we estimate that a 10 nmol/l higher 25(OH)D is similar to approximately 1 year of current smoking-related decline in FEV_1 for both ancestries, but in the opposite direction. Several putative

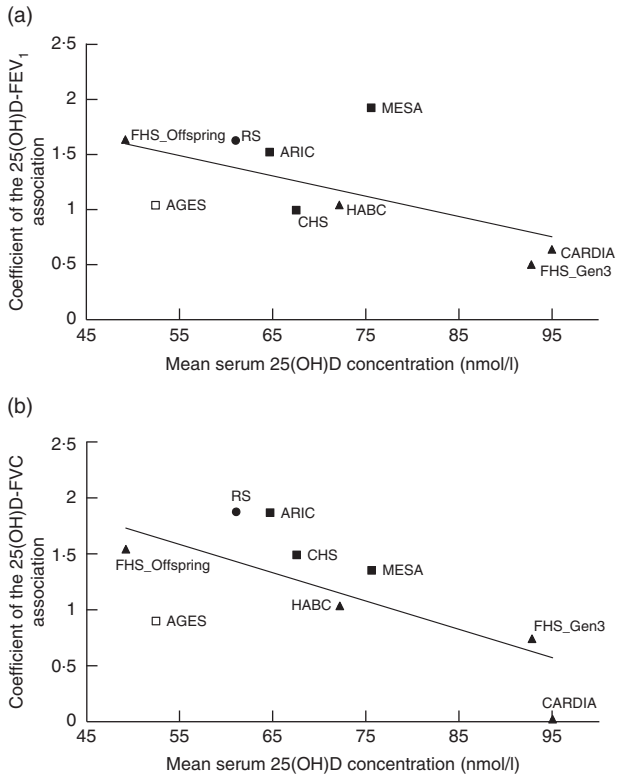

Fig. 2. Meta-regression of mean serum 25-hydroxyvitamin D (25(OH)D) levels against the association estimates of 25(OH)D with pulmonary function test in nine European ancestry cohorts in the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium. (a) Forced expiratory volume in the 1st second (FEV_1) outcome (coefficient unit: ml per 1 nmol/l 25(OH)D), and (b) forced vital capacity (FVC) outcome (coefficient unit: ml per 1 nmol/l 25(OH)D). The modifier is mean serum 25(OH)D level of each nine cohorts. A linear regression line is present for each sub-figure, with a meta-regression *P* value of 0·0006 for the FEV_1 outcome, and 0·005 for the FVC outcome. The figure also shows the measurement method for the serum 25(OH)D assay (legend shows symbols for each of the four assay methods). ■, LC-MS/MS; ▲, RIA; □, CLIA; ●, Electro-CLIA. LC-MS/MS, liquid chromatography in tandem with mass spectrometry; RIA, radioimmunoassay; CLIA, chemiluminescence immunoassay; MESA, Multi-Ethnic Study of Atherosclerosis; FHS (Offspring), Framingham Heart Study – Offspring Cohort; RS, Rotterdam Study (Netherlands); ARIC, Atherosclerosis Risk in Communities Study; AGES, Age, Gene, Environment, Susceptibility Study – Reykjavik, Iceland; CHS, Cardiovascular Health Study; HABC, Health, Aging, and Body Composition Study; CARDIA, Coronary Artery Risk Development in Young Adults Study; FHS (Gen3), Framingham Heart Study – Generation 3 Cohort.

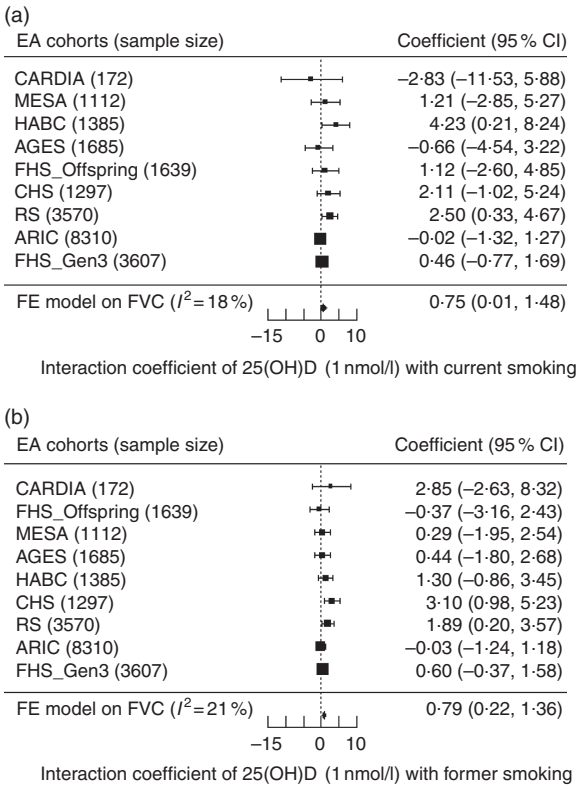

Fig. 3. Forest plots of the interaction meta-analysis of serum 25-hydroxyvitamin D (25(OH)D) and smoking status on forced vital capacity (FVC) in the European ancestry cohorts in the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium (*n* 22 777). (a) Current smokers and (b) former smokers. β (unit: ml) denotes the interaction term coefficient of 25(OH)D and smoking status on FVC from the fixed effects meta-analysis, per 1 nmol/l increment of 25(OH)D, with its 95 % CI. Cohorts were ordered from the least to the most precise, and heterogeneity is presented (I^2). EA, European ancestry; CARDIA, Coronary Artery Risk Development in Young Adults Study; MESA, Multi-Ethnic Study of Atherosclerosis; HABC, Health, Aging, and Body Composition Study; AGES, Age, Gene, Environment, Susceptibility Study – Reykjavik, Iceland; FHS (Offspring), Framingham Heart Study – Offspring Cohort; CHS, Cardiovascular Health Study; RS, Rotterdam Study (Netherlands); ARIC, Atherosclerosis Risk in Communities Study; FHS (Gen3), Framingham Heart Study—Generation 3 Cohort; FE, fixed-effects.

biological mechanisms may support a causal association between low 25(OH)D levels and worse pulmonary function. First, lung tissue cells can locally convert 25(OH)D to 1,25-$(OH)_2D$[36], the active form of vitamin D, which could improve the immune and anti-inflammatory responses in lungs via gene regulation[36–38]. If there is not enough circulating 25(OH)D, it is likely that the resolution of inflammation in lungs would be slower, which could have a negative impact on pulmonary function. In addition, 1,25-$(OH)_2D$ in lungs, converted locally from 25(OH)D, can regulate the extracellular matrix homeostasis via the ERp60-mediated pathway[39], and this is important for maintenance of lung structure. Furthermore, low vitamin D status could decrease circulating Ca status, which in turn can adversely affect thoracic skeleton mobility and respiratory muscle performance[40,41].

Our findings show that the association of serum 25(OH)D with FEV_1 and FVC were stronger in magnitude in AA *v.* EA participants, although the difference by race did not reach statistical significance. The finding may reflect the lower serum 25 (OH)D in AA participants, which is consistent with the meta-regression finding and with a previous study reporting attenuated associations at higher serum 25(OH)D[15]. Future studies that investigate genetic variation in EA and AA in the context of serum 25(OH)D may help explain the differences.

In EA participants, the positive interaction terms between serum 25(OH)D and smoking status supported a stronger magnitude of association of serum 25(OH)D with FVC in current and former smokers than in never smokers, with a consistent, but not statistically significant, difference for FEV_1. The interaction finding is consistent with a prior cross-sectional National Health and Nutrition Examination Survey (NHANES) study, which reported a stronger 25(OH)D–FEV_1 association in current and former smokers than in never smokers that was

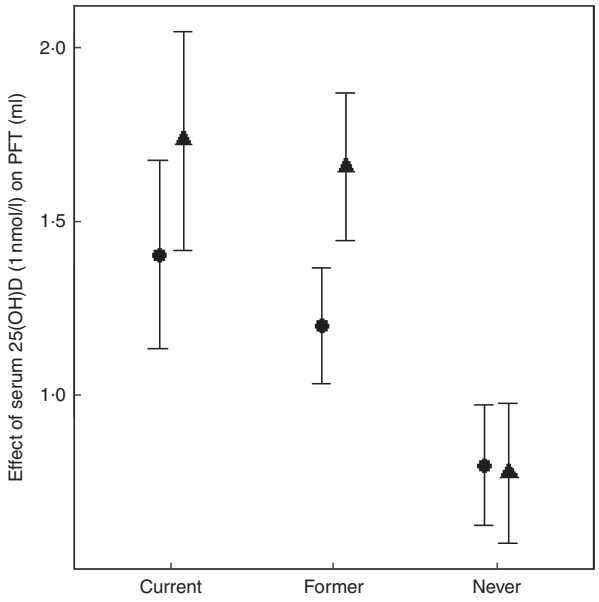

Fig. 4. Meta-analysis of the association of serum 25-hydroxyvitamin D (25(OH)D) – pulmonary function test outcomes among current, former and never smokers in the European ancestry cohorts in the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium. Forced expiratory volume in the 1st second (FEV_1, ●) and forced vital capacity (FVC, ▲) are presented for each smoking status. β (unit: ml) denotes that 1 nmol/l higher serum 25(OH)D was associated with a β mL higher FEV_1 (or FVC), calculated from an analysis including the interaction of serum 25(OH)D and smoking status. The error bar represents ±1 standard error. We used 22 786 European ancestry (EA) participants for the FEV_1 outcome and 22 777 EA participants for the FVC outcome.

near statistical significance ($P = 0{\cdot}06$)[13]. Given smokers have a higher level of oxidative stress and lower pulmonary function than never smokers, partly due to chronic inflammation in lung tissue, the stronger protective association of 25(OH)D on pulmonary function in smokers suggests a benefit for smokers. To explore this interaction, estimates of the 25(OH)D–PFT association were computed within each smoking category. In EA participants, the 25(OH)D–FEV_1 (or FVC) associations were statistically significant in all strata. Generally, in ever smokers of EA, the coefficients for 25(OH)D were greater for FVC than for FEV_1.

Meta-regression provided additional evidence for effect modification by smoking. The proportion of ever smokers was a significant modifier of the association of serum 25(OH)D with FEV_1 and FVC. The higher the proportion of ever smokers, the greater the 25(OH)D–PFT association. More specifically, the proportion of former smokers explained the heterogeneity in the 25(OH)D–PFT association across cohorts more fully than the proportion of current smokers; this may be explained by a survival bias in older participants who were current smokers. The meta-regression, based on mean age of the cohorts, showed that cohorts with a higher mean age had a greater association magnitude of 25(OH)D with FEV_1. Given that meta-regression analysis uses cohort-level factors (e.g. mean age rather than age of each individual), ecological bias is possible[42]. Nevertheless, the age-related meta-regression finding was consistent with a prior NHANES study that showed the association of 25(OH)D and FEV_1 was stronger in people over age 60 compared with younger individuals[13].

Several methodological considerations should be taken into account in interpreting the findings of this study. First, the meta-regression showed stronger 25(OH)D–PFT associations in cohorts with lower mean serum 25(OH)D, indicating a non-linear 25(OH)D–PFT association. This finding is consistent with a prior study in the FHS cohort, which reported a non-linear association and a stronger 25(OH)D–FEV_1 association in participants at risk of vitamin D deficiency (<30 nmol/l)[15]. Second, serum 25(OH)D was measured by four different methods across the cohorts. For example, two cohorts with high mean 25(OH)D (>90 nmol/l) used RIA methods. These same cohorts had a lower magnitude estimate of the 25(OH)D–PFT association; if the higher mean represents the 'truth' (and is not caused by measurement error in the RIA assay), then the lower 25(OH)D–PFT association may be primarily driven by the vitamin D distribution and not by the RIA method. Whether the assay method itself directly influences the estimate of the 25(OH)D–PFT association requires further data. Third, in this cross-sectional meta-analysis, minor differences were found in the time separation between the measurement of serum 25(OH)D and pulmonary function, but the meta-regression test for heterogeneity confirmed that time separation between measurements did not affect the 25(OH)D–PFT associations. Indeed, past studies with longitudinal measurements of serum 25(OH)D reported a high correlation of 25(OH)D measurements over a long period of time, with a correlation coefficient of 0·7 for measurements separated by 1 year, 0·5 for measurements separated by 5 years[43], and 0·42–0·52 for measurements separated by 14 years[44], which supports the use of a single 25(OH)D measurement to represent usual level. Fourth, residual confounding was unlikely given the consistent results across multiple cohorts in various settings. Weight was adjusted for the FVC outcome, given that higher weight and adiposity negatively affects lung volume (i.e. FVC)[45]; weight was not adjusted in the FEV_1 models, given FEV_1 is a measure of airways obstruction and not physical restriction of lung volume. Physical activity was not adjusted because it is not a confounder in estimating the serum 25(OH)D–PFT association; while physical activity is known to contribute to O_2 utilisation in lungs[46], little evidence and no biological rationale exists for a causal association of physical activity with either FEV_1 or FVC[47], which are markers for airways obstruction and lung volume, respectively. Finally, even though three cohorts (AGES, CARDIA, MESA) had the outcome or the exposure only measured in an ancillary study (random subset of the entire cohort), we do not expect selection bias to affect the estimate of the serum vitamin D–PFT association in this meta-analysis; indeed, the association magnitude and direction was consistent across all cohorts, regardless of the proportion of the original cohort contributing to the analysis. Thus, selection bias is expected to be negligible and would likely lead to an underestimated association, given the participants retained in the cohorts are expected to be, on average, healthier than those who were lost to follow-up.

This study meta-analysed the serum 25(OH)D–PFT association across nine cohorts, according to a common pipeline that harmonised the variables and statistical analysis. The sample size comprised 17 569 EA participants from the USA;

5269 EA participants from Iceland and the Netherlands; and 4290 AA participants from the USA, all of whom were 19–95 years old. The sample provided excellent representation of the US population, based on comparisons of demographic factors including sex, height, weight, smoking status and COPD prevalence (about 6·1 %) to national surveys[48–50], which strengthens the external validity of the study's findings.

In summary, using meta-analysis, we estimated a positive association of serum 25(OH)D with the pulmonary function parameters FEV_1 and FVC in both EA and AA participants. Associations varied by smoking status in the EA group, with stronger serum 25(OH)D–PFT associations seen in current and former smokers. The observational design means we cannot infer a causal association, and future studies, such as randomised controlled trials or Mendelian randomisation studies, are needed to further investigate the causality of 25(OH)D on pulmonary function.

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P. A. C., D. B. H. and J. X. conceived and designed the study. R. G. B., J. L., J. D., S. A. G., L. L., S. J. L., K. E. N., A. V. S., B. M. P. and L. M. S. provided the data and supervised the data analysis in each cohort. J. X., T. M. B., R. R. R., A. V. S., A. W. M., F. S., N. T. and X. Z. analysed data within each cohort. J. X., P. A. C. and D. B. H. meta-analysed and interpreted the data, co-wrote and edited the first draft of the manuscript and had primary responsibility for final content. All authors provided data, analytic support and/or study design suggestions at all stages, critically reviewed the manuscript and read and approved the final version.

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Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114518002180

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Supplemental Methods

Statistical Analysis in Individual Cohorts

The covariates smoking status, pack years, height, height squared, age, age squared, and sex were adjusted to account for variability in the pulmonary function outcomes in order to investigate other associations that are not mediated through these factors, following common practice[1, 2]. The season variable is a potential confounding factor given that serum 25(OH)D varies with seasonality[3] and that pulmonary function may be influenced by season as well[4]. In each cohort, a preliminary model with all the covariates, excluding the serum 25(OH)D term, was conducted to identify residual outliers, for each pulmonary function test (PFT) outcome. Given this, the sample size used in the subsequent primary analyses of 25(OH)D on PFT and secondary analyses that included 25(OH)D × smoking interaction may vary across the outcomes. The cutoff value for the studentized residual outliers was set at either 3 or 4, decided by each cohort separately.

Meta-Analysis

Each cohort provided a results file that included: coefficient and standard error of the association of serum 25(OH)D with each PFT outcome in the primary models, and similarly the coefficients and standard errors of the serum 25(OH)D term [a], the 25(OH)D × current smoker term [b], the 25(OH)D × former smoker term [c], and the covariance between [a] and [b] and between [a] and [c] in the interaction models.

Besides the primary meta-analysis of serum 25(OH)D on PFTs, meta-analysis of the interaction terms of 25(OH)D and smoking status (current smokers and former smokers) was conducted, with never smokers as the reference. Significant meta-analyzed coefficients of the interaction terms were further explored by a meta-analysis

of the serum 25(OH)D–PFT associations within each smoking status. The association of serum 25(OH)D with each PFT outcome among people with different smoking status in each cohort was computed from the requested parameter coefficients, standard errors, and covariance values, with example equations shown below.

$$\beta_{25(OH)D\ among\ current\ smokers} = \beta_{25(OH)D\ among\ never\ smokers/reference} + \beta_{interaction\ of\ 25(OH)D\ and\ current\ smokers}$$

$$Var_{25(OH)D\ among\ current\ smokers} = Var[1]_{25(OH)D\ among\ never\ smokers/reference}$$

$$+ Var[2]_{interaction\ of\ 25(OH)D\ and\ current\ smokers} + 2 \times Cov([1], [2])$$

Fixed-effects models were used for all the meta-analysis. Random-effects models were also tested and findings were similar to the findings generated by the fixed-effects models due to little heterogeneity (data not shown). For findings with moderate heterogeneity, meta-regression was conducted to explore the potential causes of heterogeneity.

Sensitivity analysis was conducted to assess whether the family structure within the Offspring and Gen3 cohorts in the Framingham Heart Study (FHS) would affect the meta-analysis results.

Supplemental Table 1. Flowchart of sample size dynamics in each cohort in the CHARGE Consortium, stratified by ancestry[*]

European Ancestry Cohort	**ARIC**	**CARDIA**	**CHS**	**HABC[†]**	**MESA[‡]**	**AGES[§]**	**FHS[‖]**	**RS[¶]**
Original sample size	11,478	2,478	4,346	1,794	2,501	5,519	9,219	9,895
No PFT excluded (outcome of interest)	-47	-538	-980	-117	-1,119	-2,672	-1,485	-2,542
Unacceptable PFT excluded	-9	0	-415	-22	0	-16	NA	-2,774
No height excluded	0	-10	-43	0	0	0	-1	-1
No sex excluded	0	0	0	0	0	0	0	0
No age excluded	0	0	0	0	0	0	0	0
No smoking status excluded	-7	0	-63	-2	-12	-63	-2	-6
No pack-years excluded	-130	0	-71	-27	-51	-27	-178	-172
No site excluded (if applicable)	0	0	0	0	0	0	0	0
No genetic data excluded[**]	-1,947	0	-786[††]	-124	0	-1,056	-695	-590
No serum 25(OH)D excluded (exposure of interest)	-1,011	-1,758[‡‡]	-683	-90	-198	0	-1609	-218
Unacceptable 25(OH)D excluded	0	0	0	-1	0	0	0	0
No season excluded	0	0	0	0	0	0	0	-8
No weight excluded (for the FVC analysis only)	-7	0	0	-26	0	0	0	0
Sample size for the FEV_1 analysis[§§]	***8,301***[‖‖]	***172***	***1,297***	***1,409***	***1,110***	***1,685***	***5,239***	***3,574***
Sample size for the FVC analysis[§§]	***8,310***	***172***	***1,297***	***1,385***	***1,112***	***1,685***	***5,246***	***3,570***
Sample size for the FEV_1/FVC analysis[§§]	***8,273***	***172***	***1,297***	***1,379***	***1,107***	***1,685***	***5,234***	***3,581***

African Ancestry Cohort	**ARIC**	**CARDIA**	**CHS**	**HABC**[†]	**MESA**[‡]
Original sample size	4,266	2,637	885	1,281	2,575
No PFT excluded (outcome of interest)	-80	-823	-262	-95	-1,646
Unacceptable PFT excluded	-3	0	-122	-59	0
No height excluded	0	-7	-3	0	0
No sex excluded	0	0	0	0	0
No age excluded	0	0	0	0	0
No smoking status excluded	-8	-1	-5	-2	-17
No pack-years excluded	-129	0	-22	-12	-37
No site excluded (if applicable)	0	0	0	0	0
No genetic data excluded[**]	-1,280	0	-49[††]	-117	0
No serum 25(OH)D excluded (exposure of interest)	-427	-1,649[‡‡]	-248	-132	-104
Unacceptable 25(OH)D excluded	0	0	0	-1	0
No season excluded	0	0	0	0	0
No weight excluded (for the FVC analysis only)	-2	0	0	-42	0
Sample size for the FEV_1 analysis[§§]	***2,335***	***156***	***168***	***863***	***760***
Sample size for the FVC analysis[§§]	***2,334***	***156***	***168***	***821***	***760***
Sample size for the FEV_1/FVC analysis[§§]	***2,327***	***156***	***168***	***815***	***759***

Abbreviations: 25(OH)D, 25-Hydroxyvitamin D; AGES, Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC, Atherosclerosis Risk in Communities Study; CARDIA, Coronary Artery Risk Development in Young Adults Study; CHS, Cardiovascular Health Study; FEV_1, Forced Expiratory Volume in the First Second; FHS, Framingham Heart Study; FVC, Forced Vital Capacity; HABC, Health, Aging, and Body Composition Study; MESA, Multi-Ethnic Study of Atherosclerosis; PFT, Pulmonary Function Test; RS, Rotterdam (Netherlands) Study.

* The final sample size of each cohort for each outcome variable is shown in the last three rows for each ancestry (n = 27,069 for the FEV_1 outcome, n = 27,016 for the FVC outcome, and n = 26,953 for the FEV_1/FVC outcome).

† Flowcharts of sample size in Health ABC vary slightly for different outcomes and the flowchart for FEV_1 is presented in the table.

‡ In MESA, PFT was measured only in a random sample of the entire cohort (n = 3,893 out of 5076)[5]. In addition, the final sample size for each outcome additionally excluded participants who were related genetically (n = 8 for EAs, n = 8 for AAs).

§ In AGES, spirometry was only conducted in 2002-2004 for the purpose of prioritizing the time-consuming protocol (N≈3,000)[6, 7]. And 3,219 out of the original sample size (n = 5,519) had their genetic data available[8].

‖ The flowchart of sample size in FHS has combined participants in the Offspring cohort and the Generation 3 cohort. In the Offspring cohort, only 1972 out of 5124 participants attended the 6th (1995-1998) or 7th exam (1998-2001) and had 25(OH)D measured[9], while 25(OH)D measure was planned in Exam 1 for the entire Generation 3 cohort. Given FHS only has participants with acceptable PFT measures, the exclusion of unacceptable PFT is not applicable here. Serum 25(OH)D measures that were concurrent or within 5 years of PFT measures were included.

¶ In RS, the spirometry was introduced to the cohort in 2002. At that time, the total number of participants visiting the research center was 9,895.

** Participants who did not have genetic data were excluded for the purpose of consistency and comparison with future gene by serum vitamin D meta-analysis.

†† In CHS, only those who did not have cardiovascular diseases at baseline, had available DNA, and consented to genetic testing had genetic data available (n = 3,865 out of 5,231).

‡‡ In CARDIA, serum 25(OH)D was only measured in an ancillary study of bone mineral homeostasis (n = 402). These participants were aged 24-36 years old and in good health condition[10].

§§ For each outcome, participants whose studentized residual outlier absolute value was greater than 3 (or 4), depending on each cohort, were excluded.

‖‖ In ARIC, for the FEV_1 analysis, the number of participant used for the primary analysis of serum 25(OH)D on PFT was 8,301 while the sample size for the secondary interaction analysis was 8,300. All other cohorts have consistent sample sizes for analysis of each outcome (e.g. n for primary analysis equals n for secondary interaction analysis).

Supplemental Table 2. Individual cohort descriptions, including detailed description of spirometry and serum 25(OH)D measurements

Cohort	Country	Study baseline	Spirometry	Serum 25(OH)D
AGES	Iceland	2002	Spirometry was conducted on participants in a sitting position with a disposable mouthpiece, using a Vitalograph Gold Standard Plus (Vitalograph Ltd., Buckingham, UK). The spirometer was calibrated with 1L syringe routinely. A technician explained the details before testing. The pulmonary function test was successful if there were at least two acceptable maneuvers, which was defined as no more than 300mL difference between the two attempts for at least 6 seconds in each blow. Spirometry testing was only conducted in the first 2 years, and pre-bronchodilation testing was collected[7].	Fasting blood samples were collected in September 2002 to January 2008. The serum was stored on-site at −70°C in the Clinical Biochemistry Laboratory Holtasmára at the Icelandic Heart Association[11]. The serum 25(OH)D, including D_2 and D_3, was quantified by a direct, competitive chemiluminescence immunoassay by using the LIAISON 25(OH)D Total assay (DiaSorin, Inc., Stillwater, Minnesota). The interassay coefficient of variation (CV) was 6.5% if using a previous serum pool as a control sample, and 12.7% if using the Liaison quality controls[12].
ARIC	USA	1987-9	Spirometry was conducted using a Collins Survey II water-seal spirometer (Warren E. Collins Inc., Braintree, MA) at visit 1 and 2 while SensorMedics model 1022 dry rolling seal spirometers (OMI, Houston, TX) was used at visit 5. The spirometer was calibrated daily and there was one single pulmonary function reading center to standardize the spirometry testing across the four study sites	Fasting blood samples were collected in visit 2 (1990-1992). The serum and plasma samples were stored at −80°C till 2012-2013 when the serum vitamin D was measured. The serum 25(OH)D, including D_2 and D_3, was quantified by liquid chromatography in tandem with high-sensitivity mass spectrometry (Waters Alliance e2795; Waters, Milford, MA). The CV was 10.9% using

			in ARIC. The test was successful if there were three acceptable attempts[13].	duplicate serum 25(OH)D samples at visit 2, which contained variation in both laboratory method and sample processing[14].
CARDIA	USA	1985	At year 0, 2, 5, and 10, the pulmonary function test was performed on participants by the Collins Survey 8-liter water sealed spirometer with the Eagle II microprocessor (Warren E. Collins Inc., Braintree, MA). At year 20, a dry rolling-seal SensorMedics model 1022 OMI spirometer (Viasys, Yorba Linda, CA). At year 0, 2, 5, the 1979 American Thoracic Society (ATS) guidelines was used[15]; at year 10, the 1987 ATS guideline was used[16]; while at year 20, the 2005 ATS/European Respiratory Society (ERS) criteria was used[17]. Accuracy of the spirometers was confirmed by the Pulmonary Waveform Generator (MH Custom Design and Manufacturing, Midvale, UT). The change of spirometers resulted in very slight difference in measurement for FEV_1 and FVC (6mL and 21mL, respectively). The change in spirometry guidelines resulted in 47mL and 110mL lower in FEV_1 and FVC[18].	The serum 25(OH)D was quantified by a radioimmunoassay (RIA) at the Calciotropic Hormone Reference Laboratory in the University of California, San Francisco[10].
CHS	USA	1987	The pulmonary function test was conducted at year 2, 6, and 9 in CHS, using a water-sealed spirometer (Collins Survey, Collins Medical, Inc., Braintree, MA) with accuracy	The serum samples were stored at −70°C at the Laboratory for Clinical Biochemistry Research in the University of Vermont by using methods that have

			validated, according to contemporary ATS criteria[19].	shown stability of serum biomarkers over a long period of time. The serum 25(OH)D, including D_2 and D_3, was quantified by high-performance liquid chromatography and tandem mass spectrometry on a Waters Quattro micro mass spectrometer (Waters, Milford, MA). The interassay CV was < 3.4%[20].
FHS (Offspring)	USA	1971	Pulmonary function test was conducted at each examination, adhering to the 1994 ATS criteria[21]. For the 5th, 6th, and 7th examinations of the offspring cohort, pulmonary function was measured using a 6-L water-filled Collins survey spirometer (Warren E. Collins Inc., Braintree, MA), connected to an S&M Instruments software (Doylestown, PA)[22]. Spirometer was calibrated daily[22].	Fasting blood samples of the Offspring cohort were collected in 1997-2001 (Exam 5, 6, and 7)[23]. The plasma/serum samples of the Offspring cohort were stored at −70°C till analysis[24]. The serum 25(OH)D was quantified by a radioimmunoassay (RIA, DiaSorin Inc., Stillwater, MN)[23, 25]. The serum vitamin D was log transformed in the analyses. The CV was 8.5% for a 25(OH)D control of 36nmol/L and 13.2% for a 25(OH) control of 137nmol/L[23] in the Offspring cohort[26]. The blood samples were analyzed after 1998 for serum 25(OH)D.
FHS (Gen3)	USA	2002	Pulmonary function test was conducted at each examination, adhering to the 1994 ATS criteria[21]. For the 1st and 2nd examinations of the Generation 3 cohort, pulmonary function was measured by a dry rolling-seal spirometer, connect to the CPL System	Fasting blood samples of the Generation 3 cohort were collected in 2001-2005 (Exam 1 and 2)[25, 26]. The storage temperature for the 3rd Generation cohort was −80°C[24]. The serum 25(OH)D was quantified by a radioimmunoassay (RIA, DiaSorin Inc.,

			(Warren E. Collins Inc., Braintree, MA)[22]. Spirometer was calibrated daily[22].	Stillwater, MN)[23, 25]. The serum vitamin D was log transformed in the analyses. The CV was 12.5% in the 3rd Generation cohort[26]. The blood samples were analyzed after 1998 for serum 25(OH)D. There might be small assay performance drift in FHS but the change should have been relatively small (the difference of mean 25(OH)D in 2003-2004 and 2005-2006 U.S. National Health and Nutrition Examination Survey was 2.5-5nmol/L[27]).
HABC	USA	1997	Pulmonary function testing was conducted using a horizontal dry rolling seal spirometer (SensorMedics Corporation, Yorba Linda, CA) at baseline, year 4, year 7 and year 9. The spirometers were adjusted at the National Institute of Occupational Safety and Health (Morgantown, WV), and connected to a software used in the 3rd National Health and Nutrition Examination Survey. The spirometers were calibrated daily by trained technicians, using a 3-L syringe. Starting at year 8, the EasyOne Model 2001 diagnostic spirometer (ndd Medizintechnik AG, Zurich, Switzerland) was used in home visits. These two types of spirometers were compared and both produced very similar results. Participants were asked to give at least 3 maneuvers to at most 5 maneuvers. For	Fasting blood samples were collected and stored at −80°C in Year 2. The serum 25(OH)D was quantified by a 2-step radioimmunoassay method (25-hydroxyvitaminD 125I RIA kit; DiaSorin, Inc., Stillwater, MN). The lab has met the quality criteria established by the Vitamin D External Quality Assessment Scheme, whose purpose is to ensure the analytical reliability of serum 25(OH)D assays. The interassay CV was 6.8%[29].

			participants with bronchodilator medication, their post-bronchodilator tracings were collected. All results have been reviewed centrally. A five-point quality score was created for each FVC, FEV_1, and PEF (peak expiratory flow) of each individual[28]. The reproducibility and acceptability of the pulmonary measure were based on the ATS criteria[21]. Acceptable PFT in this paper needs to have a score of 1 or greater to reduce selection bias of healthy participants.	
MESA	USA	2000	Pulmonary function test was conducted using a dry rolling seal spirometer, connected to an automated quality control software (Occupational Marketing, Inc., Houston, TX), in accordance with the 2005 ATS/ERS criteria[17]. Each participant had to have at least 3 acceptable maneuvers. A 5-point quality score was created based on a version of the National Lung Health Education Program. A quality score lower than C was viewed as low. All results have been reviewed centrally[30].	The fasting blood samples were collected and stored at −80°C at baseline in 2000-2002. In 2011-2012, the samples were shipped to University of Washington for serum 25(OH)D measurement[31]. The serum 25(OH)D level was stable during the long-term storage with a temperature of −80°C[32]. The serum 25(OH)D, including D_2 and D_3, was quantified by high-performance liquid chromatography in tandem with mass spectrometry, and calibrated by National Institute of Standards and Technology's standard reference material (SRM) 972[33]. The interassay CV for 25(OH)D_3 was 4.4% at 10.4ng/mL with a minimum detection of 2.0ng/mL, and the interassay CV for

				25(OH)D_2 was 4.4% at 9.4ng/mL with a minimum detection of 0.5ng/mL[31].
RS	Netherlands	1990	Spirometry was conducted in 2002-2009 using a SpiroPro® portable spirometer (Erich Jaeger GmbH, Hoechberg, Germany), connected to a Jaeger Master Screen PFT Pro (Care Fusion, the Netherlands), since 2009. The test was done by trained technician, in accordance with the ATS/ERS criteria[34], and pre-bronchodilator results were collected. All measures were centrally assessed and validated by researchers[35].	Non-fasting blood samples were collected in 1,428 participants at the first visit and in 3,799 participants at the third visit of the RSI cohort. Among these samples, 1,323 were overlapped. Fasting blood samples were collected in 2,464 participants at the first visit of the RSII cohort (RSII-1) and in 3,420 participants at the first visit of the RSIII cohort (RSIII-1). In RSI-3, RSII-1, and RSIII-1, the serum 25(OH)D was quantified by an electrochemi-luminescense-based assay (Elecsys Vitamin D Total, Roche Diagnostics, Mannheim, Germany), with a detectable range of 7.5-175nmol/L, a sensitivity of 10nmol/L, a within-run precision of < 6.5%, and a total precision of < 11.5%[36].

Abbreviations: 25(OH)D, 25-Hydroxyvitamin D; AGES, Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC, Atherosclerosis Risk in Communities Study; ATS, American Thoracic Society; CARDIA, Coronary Artery Risk Development in Young Adults Study; CHS, Cardiovascular Health Study; CV, Coefficient of Variation; ERS, European Respiratory Society; FEV_1, Forced Expiratory Volume in the First Second; FHS (Offspring), Framingham Heart Study—Offspring Cohort; FHS (Gen3), Framingham Heart Study—Generation 3 Cohort; FVC, Forced Vital Capacity; HABC, Health, Aging, and Body Composition Study; MESA, Multi-Ethnic Study of Atherosclerosis; PFT, Pulmonary Function Test; RIA, Radioimmunoassay; RS, Rotterdam (Netherlands) Study.

Supplemental Table 3. Cohort-specific data in the CHARGE Consortium on the time of measurement (year or exam number) for primary study variables

	Serum 25(OH)D collection time	PFTs	Smoking Status	Pack-years	Height	Weight	Age
AGES	2002-2004	2002-2004	All the covariates are concurrent with PFT.				
ARIC	1990-1992 (Visit 2)	1987 (Baseline)	All the covariates are concurrent with PFT.				
CARDIA	1992 (Year 7)	1995 (Year 10)	All the covariates are concurrent with PFT.				
CHS[*]	1992-1993 (Year 5)	1993-1994 (Year 6)	1992-1993 (Year 5)[†]	1992-1993 (Year 5)[†‡]	1992-1993 (Year 5)[†]	1992-1993 (Year 5)[†]	1992-1993 (Year 5)[†]
FHS (Offspring)[§]	1995-2001 (Between Exam 6 & Exam 7)	1991-1995 (Exam 5)/ 1995-1998 (Exam 6)/ 1998-2001 (Exam 7)	All the covariates are concurrent with PFT.				
FHS (Gen 3)[‖]	2002-2005 (Exam 1)	2002-2005 (Exam 1)/ 2008-2011 (Exam 2)[¶]	All the covariates are concurrent with PFT.				
HABC	1998-1999 (Year 2)	1997-1998 (Baseline)	All the covariates are concurrent with PFT.				
MESA	2000-2002 (Exam 1)	2004-2006 (Exam 4)	All the covariates are concurrent with PFT.				
RS							
- RSI	1997-1999 (Exam 3)	2002-2004 (Exam 4)	All the covariates are concurrent with PFT.				
- RSII	2000-2001 (Exam 1)	2004-2005 (Exam 2)	All the covariates are concurrent with PFT.				
- RSIII	2006- 2008 (Exam 1)	2006-2008 (Exam 1)	All the covariates are concurrent with PFT.				

Abbreviations: 25(OH)D, 25-Hydroxyvitamin D; AGES, Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC, Atherosclerosis Risk in Communities Study; CARDIA, Coronary Artery Risk Development in Young Adults Study; CHS, Cardiovascular Health Study; FHS (Offspring), Framingham Heart Study—Offspring Cohort; FHS (Gen3), Framingham Heart Study—Generation 3 Cohort; HABC, Health, Aging, and Body Composition Study; MESA, Multi-Ethnic Study of Atherosclerosis; PFT, Pulmonary Function Test; RS, Rotterdam (Netherlands) Study.

[*] In CHS, covariates concurrent with vitamin D blood sample were used, but results were essentially the same using covariates measured in either year 5 or year 6.
[†] In CHS, height was only measured in year 5, not in year 6. The other covariates were used if they were concurrent with 25(OH)D. The results of the 25(OH)D–PFT association were similar using either covariates in year 5 (concurrent with 25(OH)D) or in year 6 (concurrent with PFT).
[‡] In CHS, the original cohort (both European and African ancestry) was enrolled in 1989 and 1990 and the additional 2nd cohort (only African ancestry) was recruited in 1992 and 1993. For

the 1st cohort, pack-years in year 5 was extrapolated from year 2; for the 2nd cohort, pack-years was measured in year 5. The extrapolation of pack-years for the 1st cohort is similar to the actual measure in year 2.

§ Serum 25(OH)D for the Offspring cohort in FHS was measured between exam 6 and exam 7. The PFT measure and other covariates were taken from the nearest exam, within 5 years.

‖ Serum 25(OH)D of the Generation 3 cohort in FHS was measured in exam 1. The PFT measure and other covariates were taken from the nearest exam, within 5 years.

¶ 5 out of 3610 participants of the Generation 3 cohort in FHS used PFT measured in Exam 2; while the rest of participants had PFT measured in Exam 1.

Supplemental Table 4. Cohort-specific model results for the interaction of vitamin D and smoking status on pulmonary function measures in the European ancestry cohorts in the CHARGE Consortium[*]

Cohort	Smoking status	FEV_1				FVC				FEV_1/FVC (in percent)			
		β[†]	SE	P-value	Sample Size	β[†]	SE	P-value	Sample Size	β[†]	SE	P-value	Sample Size
AGES	Current	-0.7485	1.6840	0.6567	1685	-0.6614	1.9780	0.7381	1685	0.0072	0.0310	0.8169	1685
	Former	0.4603	0.9715	0.6357	1685	0.4408	1.1450	0.7003	1685	0.0277	0.0179	0.1213	1685
ARIC	Current	-0.3190	0.5793	0.5819	8300	-0.0226	0.6615	0.9727	8310	0.0039	0.0081	0.6324	8273
	Former	-0.4406	0.5394	0.4141	8300	-0.0293	0.6154	0.9620	8310	0.0010	0.0075	0.8982	8273
CARDIA	Current	-0.8417	3.6792	0.8193	172	-2.8260	4.4428	0.5257	172	0.0305	0.0505	0.5465	172
	Former	3.7482	2.3157	0.1075	172	2.8457	2.7920	0.3097	172	0.0202	0.0318	0.5272	172
CHS	Current	1.3387	1.3591	0.3248	1297	2.1117	1.5962	0.1862	1297	-0.0140	0.0270	0.6216	1297
	Former	1.9700	0.9228	**0.0330**	1297	3.1044	1.0839	**0.0043**	1297	-0.0080	0.0190	0.6532	1297
FHS - Offspring	Current	-0.8214	1.6750	0.6238	1638	1.1250	1.9030	0.5545	1639	-0.0234	0.0258	0.3641	1630
	Former	0.3424	1.2530	0.7847	1638	-0.3669	1.4250	0.7968	1639	0.0171	0.0192	0.3728	1630
FHS - Gen3	Current	0.5584	0.5387	0.3000	3601	0.4636	0.6275	0.4601	3607	0.0011	0.0073	0.8830	3604
	Former	0.2937	0.4291	0.4937	3601	0.6049	0.4996	0.2260	3607	-0.0023	0.0058	0.6965	3604
HABC	Current	4.0180	1.8399	**0.0291**	1409	4.2278	2.0488	**0.0392**	1385	0.0662	0.0282	**0.0190**	1379
	Former	0.5378	0.9930	0.5882	1409	1.2954	1.1003	0.2393	1385	-0.0260	0.0151	0.0858	1379
MESA	Current	-1.2897	1.7845	0.4700	1110	1.2085	2.0719	0.5598	1112	-0.0337	0.0310	0.2763	1107
	Former	-1.3959	0.9830	0.1559	1110	0.2943	1.1453	0.7972	1112	-0.0376	0.0171	**0.0281**	1107
RS	Current	2.3328	0.9272	**0.0119**	3574	2.4990	1.1069	**0.0240**	3570	0.0230	0.0137	0.0930	3581
	Former	1.5620	0.7182	**0.0297**	3574	1.8851	0.8592	**0.0283**	3570	0.0056	0.0106	0.5994	3581

Abbreviations: AGES, Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC, Atherosclerosis Risk in Communities Study; CARDIA, Coronary Artery Risk Development in Young Adults Study; CHS, Cardiovascular Health Study; FEV_1, Forced Expiratory Volume in the First Second; FHS (Offspring), Framingham Heart Study – the Offspring Cohort; FHS (Gen3), Framingham Heart Study – the Generation 3 Cohort; FVC, Forced Vital Capacity; HABC, Health, Aging, and Body Composition Study; MESA, Multi-Ethnic Study of Atherosclerosis; RS, Rotterdam (Netherlands) Study.

[*] Smoking status includes never smokers, former smokers, and current smokers. Never smoker was set as the reference group. Therefore, the two interaction terms are serum vitamin D × current smokers, and serum vitamin D × former smokers.

[†] β(mL) for FEV_1 and FVC outcomes and β(%) for the FEV_1/FVC ratio outcome are the coefficients of the interaction term. P-values that are ≤0.05 are bolded.

Supplemental Table 5. Cohort-specific model results for the interaction of vitamin D and smoking status on pulmonary function measures in the African ancestry cohorts in the CHARGE Consortium[*]

Cohort	Smoking status	FEV_1				FVC				FEV_1/FVC (in percent)			
		β[†]	SE	P-value	Sample Size	β[†]	SE	P-value	Sample Size	β[†]	SE	P-value	Sample Size
ARIC	Current	-0.4562	1.1974	0.7032	2335	-0.7351	1.4158	0.6036	2334	0.0219	0.0193	0.2564	2327
	Former	-0.6694	1.2747	0.5995	2335	0.8071	1.5048	0.5918	2334	-0.0242	0.0206	0.2396	2327
CARDIA	Current	-2.4238	2.8172	0.3911	156	-2.6302	3.0172	0.3849	156	0.0033	0.0415	0.9361	156
	Former	-2.3553	3.8314	0.5397	156	-1.3659	4.1121	0.7403	156	-0.0078	0.0564	0.8903	156
CHS	Current	1.3974	6.4750	0.8294	168	2.5597	7.8200	0.7438	168	-0.0004	0.1360	0.9974	168
	Former	3.3138	2.9512	0.2631	168	2.8647	3.5549	0.4215	168	0.0380	0.0620	0.5418	168
HABC	Current	1.6845	1.9362	0.3845	863	1.6975	2.1895	0.4384	821	0.0204	0.0342	0.5509	815
	Former	0.4540	1.4883	0.7604	863	0.7175	1.6947	0.6721	821	0.0212	0.0265	0.4242	815
MESA	Current	-0.8285	2.1932	0.7057	760	-0.2406	2.6210	0.9269	760	-0.0076	0.0410	0.8540	759
	Former	-0.8572	1.5037	0.5688	760	0.1427	1.7966	0.9367	760	-0.0500	0.0278	0.0725	759

Abbreviations: ARIC, Atherosclerosis Risk in Communities Study; CARDIA, Coronary Artery Risk Development in Young Adults Study; CHS, Cardiovascular Health Study; FEV_1, Forced Expiratory Volume in the First Second; FVC, Forced Vital Capacity; HABC, Health, Aging, and Body Composition Study; MESA, Multi-Ethnic Study of Atherosclerosis.

[*] Smoking status includes never smokers, former smokers, and current smokers. Never smoker was set as the reference group. Therefore, the two interaction terms are serum vitamin D × current smokers, and serum vitamin D × former smokers.

[†] β(mL) for FEV_1 and FVC outcomes and β(%) for the FEV_1/FVC ratio outcome are the coefficients of the interaction term.

Supplemental Table 6. The meta-analysis of the interaction of serum vitamin D and smoking status on pulmonary function measures, stratified by ancestry, in the CHARGE Consortium[*]

	FEV_1			FVC			FEV_1/FVC (in percent)		
	β	**SE**	**P-value**	**β**	**SE**	**P-value**	**β**	**SE**	**P-value**
European ancestry[†]:									
Current vs. Never Smokers	0.4805	0.3241	0.1382	0.7452	0.3756	**0.0473**[‡]	0.0047	0.0047	0.3162
Former vs. Never Smokers	0.3725	0.2507	0.1373	0.7913	0.2908	**0.0065**[‡]	-0.0015	0.0037	0.6794
African ancestry[§]:									
Current vs. Never Smokers	-0.2369	0.8698	0.7853	-0.301	1.0105	0.7658	0.0155	0.0145	0.2851
Former vs. Never Smokers	-0.2163	0.7688	0.7784	0.6433	0.8988	0.4741	-0.0149	0.0133	0.2621

Abbreviations: FEV_1, Forced Expiratory Volume in the First Second; FVC, Forced Vital Capacity.

[*] Fixed effect models were used. β(mL) for FEV_1 and FVC outcomes and β(%) for the FEV_1/FVC ratio outcome are the coefficients of the interaction terms only (the interaction of current smoking status and serum vitamin D, and the interaction of former smoking status and serum vitamin D) with the reference group of never smokers.

[†] n = 22,786 for the FEV_1 outcome, n = 22,777 for the FVC outcome, and n = 22,728 for the FEV_1/FVC outcome in European ancestry cohorts.

[‡] P-values that are ≤0.05 are bolded.

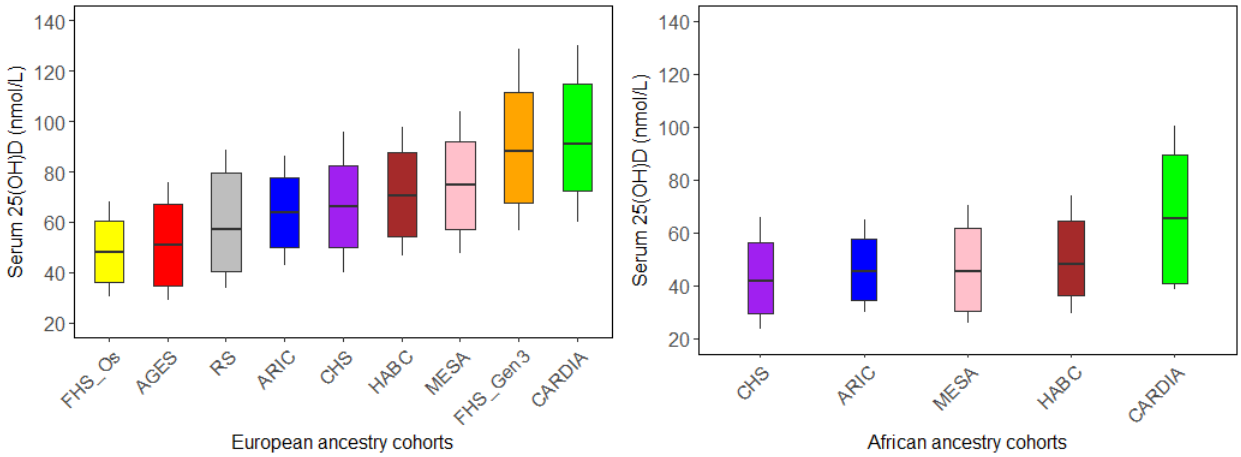
[§] n = 4,282 for the FEV_1 outcome, n = 4,239 for the FVC outcome, and n = 4,225 for the FEV_1/FVC outcome in African ancestry cohorts.

Supplemental Table 7. Cohort-specific model results for the primary analysis of vitamin D on pulmonary function measures in the CHARGE Consortium, stratified by ancestry

Cohort	Ancestry	FEV_1				FVC				FEV_1/FVC (in percent)			
		β*	SE	P-value	Sample Size	β*	SE	P-value	Sample Size	β*	SE	P-value	Sample Size
AGES	EA	1.0350	0.4696	**0.0276**	1685	0.8992	0.5570	0.1067	1685	0.0059	0.0087	0.4982	1685
ARIC	EA	1.5158	0.2422	**<.0001**	8301	1.8670	0.2816	**<.0001**	8310	-0.0090	0.0034	**0.0077**	8273
CARDIA	EA	0.6361	1.0020	0.5265	172	0.0223	1.2104	0.9853	172	0.0057	0.0137	0.6770	172
CHS	EA	0.9901	0.4476	**0.0270**	1297	1.4899	0.5355	**0.0054**	1297	-0.0150	0.0090	0.1079	1297
FHS	EA – Offspring	1.6300	0.6022	**0.0068**	1638	1.5414	0.6994	**0.0275**	1639	-0.0105	0.0093	0.2563	1630
	EA – Gen3	0.4989	0.2018	**0.0134**	3601	0.7410	0.2395	**0.0020**	3607	-0.0071	0.0027	**0.0090**	3604
HABC	EA	1.0385	0.4847	**0.0323**	1409	1.0349	0.5452	0.0579	1385	-0.0006	0.0074	0.9340	1379
MESA	EA	1.9165	0.4884	**<.0001**	1110	1.3521	0.5783	**0.0196**	1112	0.0030	0.0085	0.7270	1107
RS	EA	1.6213	0.3212	**<.0001**	3574	1.8748	0.3912	**<.0001**	3570	0.0010	0.0048	0.8400	3581
ARIC	AA	1.5913	0.5431	**0.0034**	2335	1.4779	0.6438	**0.0218**	2334	-0.0025	0.0088	0.7780	2327
CARDIA	AA	0.5740	1.3184	0.6639	156	1.1119	1.4219	0.4355	156	-0.0056	0.0194	0.7745	156
CHS	AA	1.9361	1.4718	0.1884	168	0.1999	1.7871	0.9109	168	0.0470	0.0310	0.1236	168
HABC	AA	2.0883	0.6766	**0.0021**	863	1.6116	0.7768	**0.0383**	821	0.0219	0.0120	0.0689	815
MESA	AA	2.1447	0.7209	**0.0030**	760	2.0604	0.8732	**0.0186**	760	-0.0091	0.0134	0.4960	759

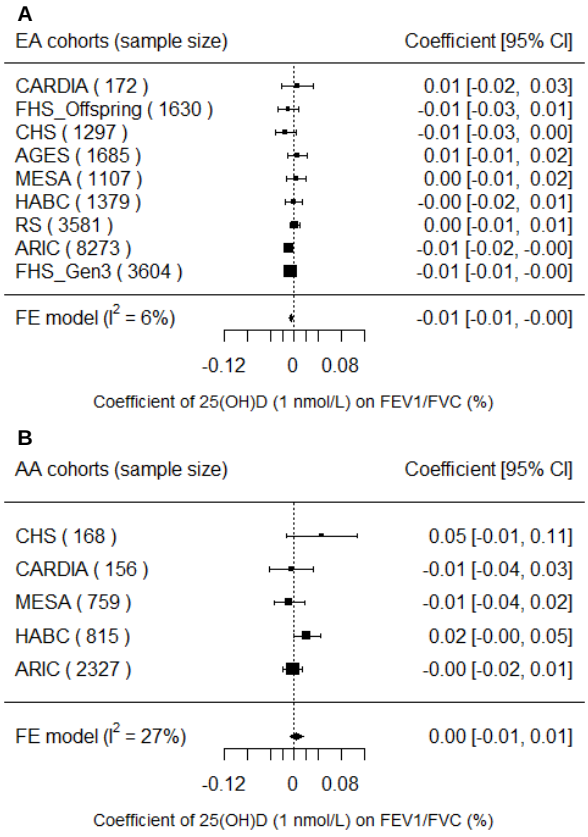
Abbreviations: AA, African Ancestry; AGES, Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC, Atherosclerosis Risk in Communities Study; CARDIA, Coronary Artery Risk Development in Young Adults Study; CHS, Cardiovascular Health Study; EA, European Ancestry; FEV_1, Forced Expiratory Volume in the First Second; FHS (Offspring), Framingham Heart Study – the Offspring Cohort; FHS (Gen3), Framingham Heart Study – the Generation 3 Cohort; FVC, Forced Vital Capacity; HABC, Health, Aging, and Body Composition Study; MESA, Multi-Ethnic Study of Atherosclerosis; RS, Rotterdam (Netherlands) Study.

* The beta coefficient corresponds to the association of serum vitamin D on the specific pulmonary function outcome (β(mL) for FEV_1 and FVC and β(%) for the FEV_1/FVC ratio). P-values that are ≤0.05 are bolded.



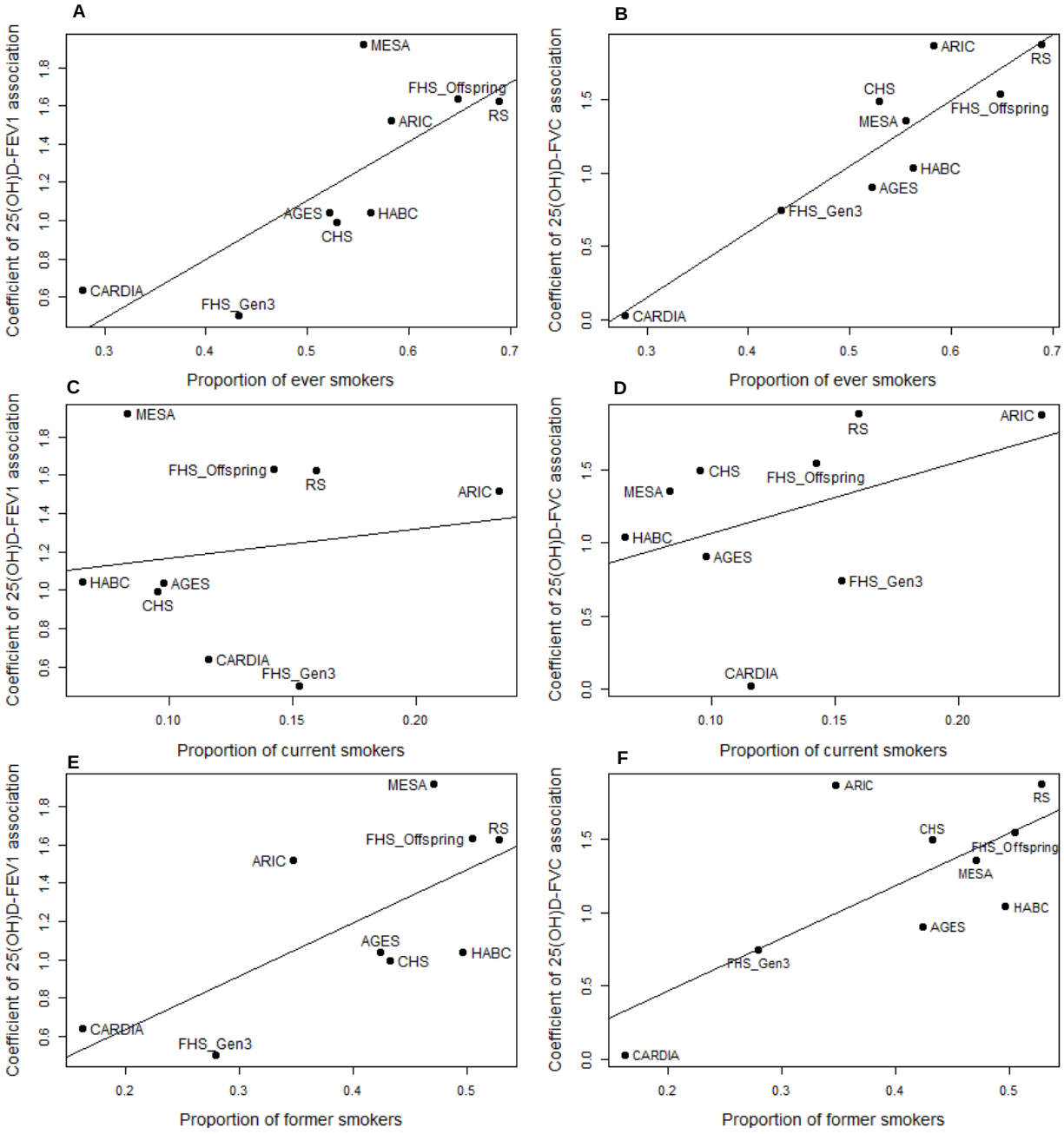
Supplemental Figure 1. Serum vitamin D distribution in each cohort in the CHARGE Consortium, stratified by ancestry (*n* = 22,838 for EAs, *n* = 4,290 for AAs). The middle bar is the median of serum vitamin D level; the lower and upper bars of the box represent the 25 and 75 percentile values of serum vitamin D; the minimum and maximum of the whisker were computed as mean - SD, and mean + SD, respectively, given that the vitamin D distribution was approximately normal in each cohort.

Abbreviations: 25(OH)D, 25-Hydroxyvitamin D; AGES, Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC, Atherosclerosis Risk in Communities Study; CARDIA, Coronary Artery Risk Development in Young Adults Study; CHS, Cardiovascular Health Study; FHS_Os, Framingham Heart Study – the Offspring Cohort; FHS_Gen3, Framingham Heart Study – the Generation 3 Cohort; HABC, Health, Aging, and Body Composition Study; MESA, Multi-Ethnic Study of Atherosclerosis; RS, Rotterdam (Netherlands) Study.



Supplemental Figure 2. Forest plots of the meta-analysis of serum 25(OH)D on FEV_1/FVC across cohorts in the CHARGE Consortium, stratified by participant ancestry. Associations are presented for serum 25(OH)D on (A) FEV_1/FVC in European ancestry cohorts (n = 22,728) and (B) FEV_1/FVC in African ancestry cohorts (n = 4,225). β (in percent) denotes the coefficient of the serum vitamin D–FEV_1/FVC association, with its 95% confidence interval; The cohort name and sample size of each cohort are specified in subpart A and subpart B; a positive association is greater than 0 while a negative association is less than 0, and fixed effect meta-analysis was used. Cohorts listed in the forest plots were ordered from the least to the most precise, and heterogeneity is presented (I^2).

Abbreviation: 25(OH)D, 25-Hydroxyvitamin D; AA, African Ancestry; AGES, Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC, Atherosclerosis Risk in Communities Study; CARDIA, Coronary Artery Risk Development in Young Adults Study; CHS, Cardiovascular Health Study; CI, Confidence Interval; EA, European Ancestry; FE, Fixed-Effects; FEV_1, Forced Expiratory Volume in the First Second; FHS (Offspring), Framingham Heart Study—Offspring Cohort; FHS (Gen3), Framingham Heart Study—Generation 3 Cohort; FVC, Forced Vital Capacity; HABC, Health, Aging, and Body Composition Study; MESA, Multi-Ethnic Study of Atherosclerosis; RS, Rotterdam (Netherlands) Study.


A
Coefficient of 25(OH)D-FEV1 association
Proportion of ever smokers
MESA
FHS_Offspring
RS
ARIC
AGES
HABC
CHS
CARDIA
FHS_Gen3
B
Coefficient of 25(OH)D-FVC association
Proportion of ever smokers
ARIC
RS
CHS
MESA
FHS_Offspring
HABC
AGES
FHS_Gen3
CARDIA
C
Coefficient of 25(OH)D-FEV1 association
Proportion of current smokers
MESA
FHS_Offspring
RS
ARIC
HABC
AGES
CHS
CARDIA
FHS_Gen3
D
Coefficient of 25(OH)D-FVC association
Proportion of current smokers
RS
ARIC
CHS
FHS_Offspring
MESA
HABC
AGES
FHS_Gen3
CARDIA
E
Coefficient of 25(OH)D-FEV1 association
Proportion of former smokers
MESA
RS
FHS_Offspring
ARIC
AGES
CHS
HABC
CARDIA
FHS_Gen3
F
Coefficient of 25(OH)D-FVC association
Proportion of former smokers
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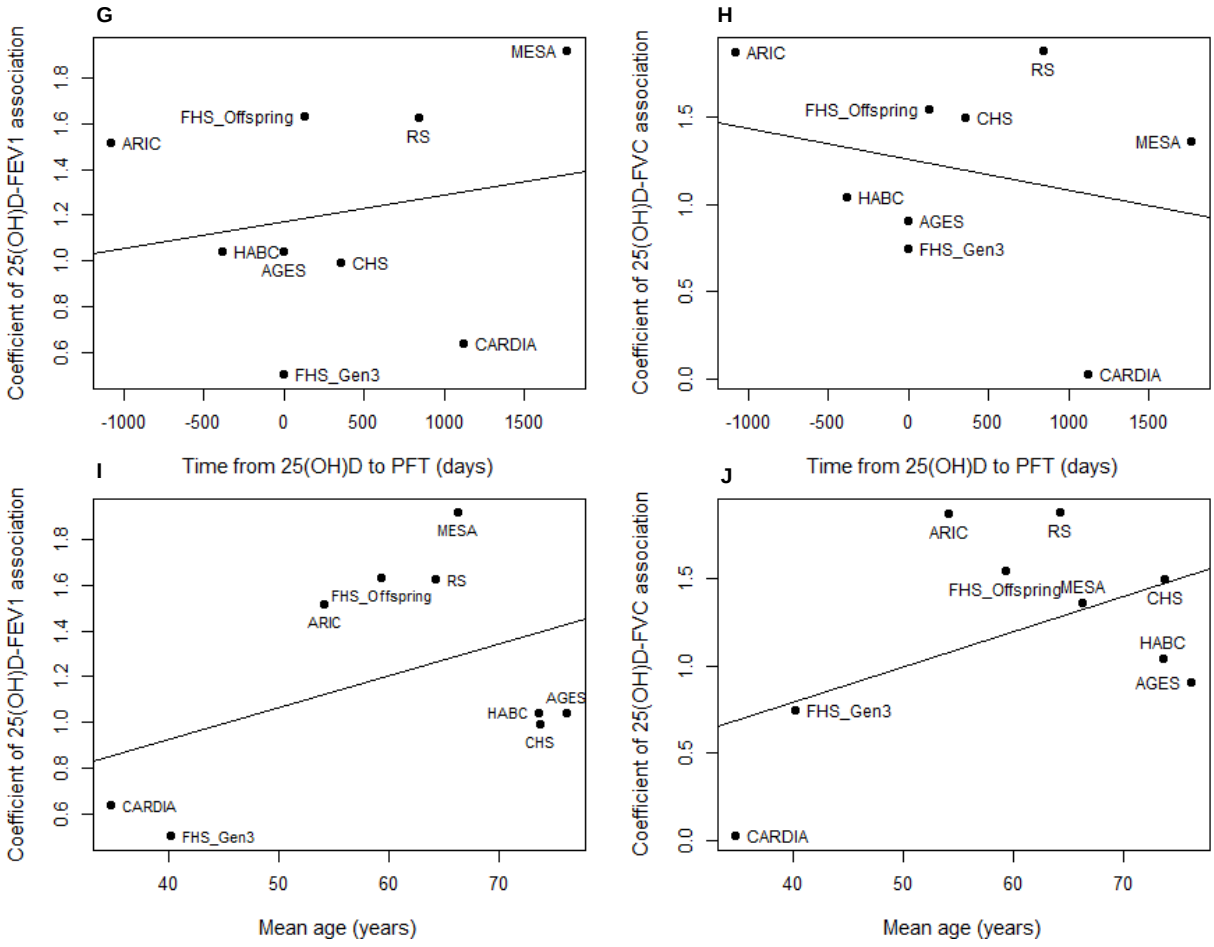
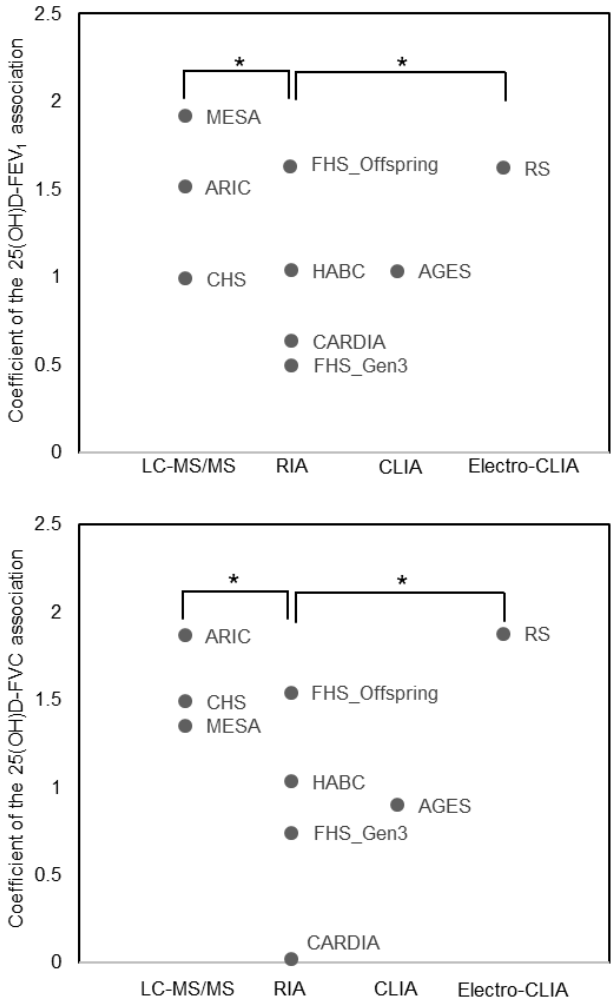
FHS_Offspring
MESA
HABC
AGES
FHS_Gen3
CARDIA



Supplemental Figure 3. Meta-regression plots of individual modifiers against FEV_1 and FVC, in nine European ancestry cohorts in the CHARGE Consortium. The modifiers include proportion of ever smokers, proportion of current smokers, proportion of former smokers, time from 25(OH)D to PFT (days), and mean age (years) of each nine cohorts, to check for between-study heterogeneity explained by each modifier. The y axis is the association coefficient (β) of 25(OH)D (per nmol/L) with PFT (FEV_1 or FVC, mL). (A) Plot of proportion of ever smokers against 25(OH)D–FEV_1 association (P = 0.0002); (B) plot of proportion of ever smokers against 25(OH)D–FVC association (P = 0.001); (C) plot of proportion of current smokers against 25(OH)D–FEV_1 association (P = 0.314); (D) plot of proportion of current smokers against 25(OH)D–FVC association (P = 0.053); (E) plot of proportion of former smokers against 25(OH)D–FEV_1 association (P = 0.002); (F) plot of

proportion of former smokers against 25(OH)D–FVC association (P = 0.037); (G) plot of measurement time interval against 25(OH)D–FEV_1 association (P = 0.727); (H) plot of measurement time interval against 25(OH)D–FVC association (P = 0.405); (I) plot of cohort mean age against 25(OH)D–FEV_1 association (P = 0.009); (J) plot of cohort mean age against 25(OH)D–FVC association (P = 0.083). The linear regression line is present in each scatter plot with a continuous modifier.

Abbreviation: 25(OH)D, 25-Hydroxyvitamin D; AGES, Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC, Atherosclerosis Risk in Communities Study; CARDIA, Coronary Artery Risk Development in Young Adults Study; CHS, Cardiovascular Health Study; FEV_1, Forced Expiratory Volume in the First Second; FHS (Offspring), Framingham Heart Study—Offspring Cohort; FHS (Gen3), Framingham Heart Study—Generation 3 Cohort; FVC, Forced Vital Capacity; HABC, Health, Aging, and Body Composition Study; MESA, Multi-Ethnic Study of Atherosclerosis; PFT, Pulmonary Function Test; RS, Rotterdam (Netherlands) Study.



Supplemental Figure 4. Meta-regression of methods of 25(OH)D measure against the association estimates of 25(OH)D with PFT in nine European ancestry cohorts in the CHARGE Consortium. The modifier is method of 25(OH)D measure in each of the nine cohorts. The method of 25(OH)D measure is a categorical variable with 4 categories (LC-MS/MS, RIA, CLIA, electro-CLIA). Pairwise comparisons of the 4 methods in the meta-regression model showed significant differences in the 25(OH)D–PFT associations between cohorts using RIA and cohorts using LC/MS/MS ($p < 0.005$), and also between cohorts using RIA and one cohort using electro-CLIA ($p < 0.02$).

Abbreviation: 25(OH)D, 25-Hydroxyvitamin D; AGES, Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC, Atherosclerosis Risk in Communities Study; CARDIA, Coronary Artery Risk Development in Young Adults Study; CHS, Cardiovascular Health Study; CLIA, Chemiluminescence Immunoassay; FEV_1, Forced Expiratory Volume in the First Second; FHS (Offspring), Framingham Heart Study—Offspring Cohort; FHS (Gen3), Framingham Heart Study—Generation

3 Cohort; FVC, Forced Vital Capacity; HABC, Health, Aging, and Body Composition Study; LC-MS/MS, Liquid Chromatography in Tandem with Mass Spectrometry; MESA, Multi-Ethnic Study of Atherosclerosis; PFT, Pulmonary Function Test; RIA, Radioimmunoassay; RS, Rotterdam (Netherlands) Study.

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CHAPTER 3

OMEGA-3 FATTY ACIDS AND GENOME-WIDE INTERACTION ANALYSES REVEAL *DPP10*-PULMONARY FUNCTION ASSOCIATION

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ORIGINAL ARTICLE

Omega-3 Fatty Acids and Genome-Wide Interaction Analyses Reveal *DPP10*–Pulmonary Function Association

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Abstract

Rationale: Omega-3 polyunsaturated fatty acids (*n*-3 PUFAs) have anti-inflammatory properties that could benefit adults with comprised pulmonary health.

Objective: To investigate *n*-3 PUFA associations with spirometric measures of pulmonary function tests (PFTs) and determine underlying genetic susceptibility.

Methods: Associations of *n*-3 PUFA biomarkers (α-linolenic acid, eicosapentaenoic acid, docosapentaenoic acid [DPA], and docosahexaenoic acid [DHA]) were evaluated with PFTs (FEV_1, FVC, and FEV_1/FVC) in meta-analyses across seven cohorts from the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium (N = 16,134 of European or African ancestry). PFT-associated *n*-3 PUFAs were carried forward to genome-wide interaction analyses in the four largest cohorts (N = 11,962) and replicated in one cohort (N = 1,687). Cohort-specific results were combined using joint 2 degree-of-freedom (2*df*) meta-analyses of SNP associations and their interactions with *n*-3 PUFAs.

Results: DPA and DHA were positively associated with FEV_1 and FVC ($P < 0.025$), with evidence for effect modification by smoking and by sex. Genome-wide analyses identified a novel association of rs11693320—an intronic *DPP10* SNP—with FVC when incorporating an interaction with DHA, and the finding was replicated ($P_{2df} = 9.4 \times 10^{-9}$ across discovery and replication cohorts). The rs11693320-A allele (frequency, ∼80%) was associated with lower FVC ($P_{SNP} = 2.1 \times 10^{-9}$; $\beta_{SNP} = -161.0$ ml), and the association was attenuated by higher DHA levels ($P_{SNP \times DHA\ interaction} = 2.1 \times 10^{-7}$; $\beta_{SNP \times DHA\ interaction} = 36.2$ ml).

Conclusions: We corroborated beneficial effects of *n*-3 PUFAs on pulmonary function. By modeling genome-wide *n*-3 PUFA interactions, we identified a novel *DPP10* SNP association with FVC that was not detectable in much larger studies ignoring this interaction.

Keywords: FEV_1; FVC; smoking; genome-wide association study; omega-3 fatty acids

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At a Glance Commentary

Scientific Knowledge on the Subject: Omega-3 polyunsaturated fatty acids (*n*-3 PUFAs) have anti-inflammatory properties that may beneficially affect pulmonary function. Few population-based studies have investigated the associations between *n*-3 PUFA biomarkers and pulmonary function, and whether smoking or sex modifies these associations. Moreover, genome-wide association studies have identified >150 genetic loci on pulmonary function, yet no studies have examined interactions between genetic variants and *n*-3 PUFAs.

What This Study Adds to the Field: We found associations of docosahexaenoic acid and docosapentaenoic acid with pulmonary function, and modifications of association by cigarette smoking and by sex. In addition, we identified a novel association in the *DPP10* gene with FVC at genome-wide significance. This *DPP10* association was not found in standard genome-wide analyses and was only discovered after incorporating the interaction with the *n*-3 PUFA biomarker levels.

Pulmonary function tests (PFTs) provide indicators of lung health and mortality risk in the general population (1). Impaired pulmonary function increases the risk of chronic obstructive pulmonary disease (COPD) (2), which is one of the leading causes of death worldwide (3, 4). PFTs include measurement of FEV_1, FVC, and FEV_1/FVC to diagnose COPD and follow its progression.

PFTs are heritable traits (~35%) (5), and genome-wide association studies (GWASs) have identified >150 PFT-associated loci (6–13). Environmental factors, including cigarette smoking that contributes to chronic inflammation (14), also influence PFTs. Omega-3 polyunsaturated fatty acids (*n*-3 PUFAs) may mitigate the inflammatory response. *n*-3 PUFAs include α-linolenic acid (ALA) and its long-chain derivatives, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA). ALA, the predominant *n*-3 PUFA in the Western diet, is present in vegetable oils; EPA, DPA, and DHA are found mainly in fish. After absorption, some dietary ALA is converted through endogenous elongation and desaturation reactions (15) to the long-chain derivatives. However, dietary ALA may not adequately replace dietary EPA, DPA, and DHA given the limited conversion rate (16). We focused on these *n*-3 PUFAs based on prior evidence that they help combat inflammation in the lung by generating lipid-derived mediators, such as resolvins (17, 18).

Diets rich in *n*-3 PUFAs have been implicated in preventing chronic inflammatory diseases, including cardiovascular disease, rheumatoid arthritis, and dementia (19). Few studies have investigated the role of *n*-3 PUFAs in lung health. Two studies investigated dietary-reported *n*-3 PUFAs with PFTs; one found that higher *n*-3 PUFAs were associated with higher PFTs (20), whereas the other reported null associations (21). One study investigating serum *n*-3 PUFAs with PFTs found positive associations of DHA with FEV_1 and FVC (22). Another study, conducted only in ever smokers, found that higher plasma DHA was associated with lower odds of COPD (23).

Tests that jointly model environmental factors and gene-by-environment interactions can identify novel genetic associations (24–26). No prior GWAS of PFTs has investigated interactions with *n*-3 PUFAs or other nutrient biomarkers. In this study, we tested the association of *n*-3 PUFA biomarkers with cross-sectional PFTs and then studied genome-wide interactions of SNPs and insertions/deletions (indels) with *n*-3 PUFAs on PFTs in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium. We combined cohort-specific results to estimate the *n*-3 PUFA-PFT associations and to identify genetic associations with PFTs when accounting for *n*-3 PUFA interactions. Preliminary results of our study, reporting *n*-3 PUFA biomarker associations with PFTs, were previously published in the form of an abstract (27).

Methods

Cohorts and Participants

Seven cohorts—AGES, ARIC, CARDIA, CHS, FHS, MESA, and RS—contributed to meta-analyses of *n*-3 PUFA-PFT associations. All cohorts included European ancestry (EA) participants; three cohorts also included African ancestry (AA) participants (Table 1). Our genome-wide interaction analyses focused on the five largest cohorts ($N > 500$). For additional cohort details, *see* online supplement and Tables E1–E3 in the online supplement. Institutional Review Boards at the respective institutions approved all data collection.

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Author Contributions: J.X., P.A.C., and D.B.H. conceived and designed the study. A.W.M., R.G.B., J.D., B.M.P., S.A.G., R.N.L., M.F., L.L., K.E.N., A.V.S., V.G., and L.M.S. provided the data and supervised the data analysis in each cohort. J.X., N.C.G., T.M.B., R.H., R.R.R., A.V.S., A.W.M., N.P., F.S., N.T., X.Z., and C.A.M. analyzed cohort-specific data and/or performed meta-analyses. M.S. mirrored the meta-analysis and confirmed the results. J.X., N.C.G., C.A.M., B.K.P., P.A.C., and D.B.H. interpreted the results. J.X., C.A.M., B.K.P., P.A.C., and D.B.H. cowrote and edited the first draft of the manuscript. All authors provided support and suggestions at all stages, critically reviewed the manuscript, and approved the final version.

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PFTs and *n*-3 PUFA Measurements

PFTs, specifically FEV_1 (ml), FVC (ml), and FEV_1/FVC (%), were measured by spirometry. *n*-3 PUFAs were measured in plasma phospholipids in all cohorts except FHS (*see* online supplement; *see* Table E4 for measurement details and Table E5 for measurement times). *n*-3 PUFAs were measured in red blood cells in FHS, which are strongly correlated with plasma measures (16, 28). In each cohort, *n*-3 PUFAs were measured as a relative percentage of total fatty acids. Both PFTs and *n*-3 PUFAs were continuous variables.

Statistical Analysis for *n*-3 PUFA Associations with PFTs

Linear regression models were run, separately by EA or AA in each cohort, to estimate *n*-3 PUFA associations with PFTs (*see* online supplement). Models were extended to include interaction terms to assess effect modification by smoking status and sex. Fixed-effects meta-analysis was used to combine cohort- and ancestry-specific estimates of *n*-3 PUFA–PFT associations and *n*-3 PUFA interactions with smoking status and sex (29). Smoking-stratified meta-analyses were also performed, and heterogeneity at the cohort level was examined (*see* online supplement).

Statistical Analysis for Genome-Wide Interaction with *n*-3 PUFAs on PFTs

Genome-wide interactions with *n*-3 PUFAs were studied using joint 2*df* meta-analyses (30) under a fixed-effects model, as done before in single ancestry (31) and cross-ancestry genome-wide meta-analyses (32). Robust standard error estimation and inverse variance weighting were applied (33), similar to the prior genome-wide variant × smoking study for PFTs in CHARGE (24). The same covariates were adjusted (*see* online supplement) along with ancestral principal components. Cohort- and ancestry-specific coefficients of SNP/indel (henceforth, collectively referred to as SNP) additive dosage (β_{SNP}) and SNP × *n*-3 PUFA interaction term ($\beta_{SNP\times n\text{-}3\ PUFA\ interaction}$) in the four discovery cohorts were combined using METAL with genomic control applied (N = 11,962; 11,165 EA participants, 797 AA participants; Table 1). The standard *a priori* level of genome-wide significance was used ($P < 5 \times 10^{-8}$) for the discovery meta-analysis (34), as done in our prior study (24). CARDIA was reserved for replication (N = 1,687; 1,141 EA participants, 546 AA participants; Table 1). The threshold for declaring significance in the replication phase was 0.05 given that only one SNP in one model was tested. To characterize top SNP findings, we pursued three additional analyses across the discovery and replication cohorts: smoking-stratified and sex-stratified joint 2*df* meta-analyses to examine selected SNP × *n*-3 PUFA interactions; and standard 1*df* meta-analyses to assess evidence of SNP associations without considering *n*-3 PUFA interaction.

Bioinformatics Analysis

Follow-up analyses were conducted to assess SNP regulatory potential and gene function. *In silico* analyses used HaploReg v4.1 (35), Roadmap Epigenomics (36), Genotype-Tissue Expression Project (GTEx, version 7) (37), and GeneMANIA (38) (*see* online supplement).

Results

Characteristics of cohort participants and their *n*-3 PUFA distributions are provided in Table 1 (*see* additional details online supplement, Table E6, and Figure E1). There was little correlation ($|r| < 0.2$) between the *n*-3 PUFAs (ALA, EPA, DPA, or DHA) with pack-years, and average levels were similar across smoking strata, except for DHA, which showed a relatively consistent pattern across cohorts with the highest levels in never smokers, followed by former smokers, and then current smokers (Table E7).

Meta-analysis of Associations of *n*-3 PUFAs and Interactions with Smoking and Sex on PFTs

For FEV_1 and FVC, cross-ancestry meta-analyses revealed positive associations of DHA and DPA at $P < 0.05$ (Figures 1, E2, and E3 and Table E8). To convey the impact of differences in *n*-3 PUFA levels, we estimated that 1 SD higher DHA (~1.3% of plasma total FAs) was associated with 18.6-ml higher FEV_1 ($P = 6.1 \times 10^{-6}$) and 10.9-ml higher FVC ($P = 0.02$) and that 1 SD higher DPA (~0.2% of plasma total FAs) was associated with 7.9-ml higher FEV_1 ($P = 0.0006$) and 6.5-ml higher FVC ($P = 0.01$) (for sensitivity analysis, *see* online supplement). A positive association was also indicated between ALA and FVC: 1 SD higher ALA (0.07% of plasma total FAs) was associated with 8.4-ml higher FVC ($P = 0.023$). This ALA finding was mainly driven by AA (Table E8).

Smoking status significantly modified the DHA–FEV_1 association ($P_{DHA\times current\ smoking\ interaction} = 0.02$; Figure E4) such that the magnitude of the association was larger in current smokers. To further interpret the DHA interaction with smoking status on FEV_1, β coefficients for current, former, and never smokers were estimated ($\beta_{DHA} + \beta_{DHA\times smoking\ interaction}$). Across all cohorts, a 1% higher DHA was associated with a 39-ml higher FEV_1 ($P = 0.0001$) in current smokers; this effect size was about threefold the magnitude observed for former (13 ml; $P = 0.010$) and never smokers (11 ml; $P = 0.012$) (Figure 2).

For FEV_1/FVC, the cross-ancestry and EA-specific meta-analyses revealed associations with EPA and ALA ($P < 0.02$) and with DHA among current smokers ($P < 0.0001$) (Figure E4). However, because the effect sizes were small (<1% increase in FEV_1/FVC with 1 SD higher *n*-3 PUFA; Tables E8 and E9), further analyses focused on FEV_1 and FVC. No significant interaction of smoking status with DPA was observed for any PFT outcome ($P = 0.06$–0.35).

Sex was an effect modifier in the DPA–FVC association ($P_{DPA\times sex\ interaction} = 0.035$) such that the magnitude of the DPA association was larger in males (1 SD [~0.2%] higher DPA had a greater association with FVC [by 10 ml] in males than in females). Sex modification, however, was not observed for DPA–FEV_1, DHA–FEV_1, or DHA–FVC associations ($P_{interaction} = 0.17$–0.83).

Genome-Wide Interaction Analyses of *n*-3 PUFAs on PFTs

DHA and DPA were carried forward to genome-wide interaction analyses because each was associated at $P < 0.05$ in both the EA-specific and the cross-ancestry meta-analyses. Because the ALA association was primarily driven by AA participants, which represented a small portion of the total sample size used for the *n*-3 PUFA-PFT analysis (~16%), ALA was not carried forward. Genome-wide joint 2*df* interaction analyses with *n*-3 PUFAs captured 7.2 million genotyped and 1000 Genomes–imputed SNPs with minor allele frequency >5% across 11,165 EA participants and 797 AA participants (Table 1). There was no indication of genomic inflation bias ($\lambda_{gc} = 1.02$–1.03; Figures E5–E8).

Table 1. Participant Characteristics in All Seven Cohorts (*N* = 16,134; 13,629 EA Participants and 2,505 AA Participants)

	n-3 PUFA Biomarkers on PFT Measures										
	Discovery Phase of Genome-Wide Interaction Analysis of *n*-3 PUFA Biomarkers									Replication Phase: CARDIA	
			CHS*			FHS		MESA			
	AGES (EA)	RS (EA)	AA	EA	ARIC (EA)	Offspring (EA)	Gen3 (EA)	AA	EA	AA	EA
N for *n*-3 PUFA-PFT association†	424	141	243	1,690	3,254	2,169	3,052	801	1,140	1,461	1,759
N for genome-wide interaction‡	NA§	NA§	NA§	1,684	3,143	5,198‖		797	1,140	546¶	1,141¶
Males, %	45.0	48.9	30.9	39.1	48.5	45.0	47.2	47.7	49.6	39.3	46.5
Age, yr	76.3 (5.5)	74.6 (5.7)	72.9 (5.2)	74.5 (4.8)	53.8 (5.6)	66.0 (8.9)	46.0 (8.8)	65.6 (9.7)	66.4 (9.9)	44.6 (3.8)	45.8 (3.4)
Height, m	1.67 (0.09)	1.68 (0.09)	1.64 (0.08)	1.64 (0.09)	1.69 (0.09)	1.67 (0.10)	1.70 (0.09)	1.68 (0.10)	1.69 (0.10)	1.70 (0.10)	1.72 (0.09)
Weight, kg**	76.4 (14.8)	77.9 (13.7)	77.4 (13.0)	71.3 (13.8)	77.9 (16.2)	79.3 (17.6)	81.1 (19.3)	84.8 (17.2)	79.8 (17.5)	90.5 (23.0)	81.8 (19.5)
Current smokers, %	11.1	14.2	12.3	8.4	22.8	8.0	9.8	15.5	8.7	24.1	14.3
Former smokers, %	40.1	57.5	42.0	48.5	39.5	53.0	32.2	38.6	47.1	13.5	24.4
Pack-years††	24.6 (17.5)	27.0 (23.5)	25.4 (26.0)	28.7 (25.2)	26.8 (19.7)	24.6 (21.7)	13.1 (14.3)	24.6 (23.5)	29.7 (29.1)	10.8 (9.2)	12.2 (12.5)
FEV_1, ml	2,176 (664)	2,387 (748)	1,765 (482)	2,036 (614)	3,080 (780)	2,630 (767)	3,388 (773)	2,189 (662)	2,555 (761)	2,704 (684)	3,329 (753)
FVC, ml	2,920 (815)	3,238 (952)	2,440 (694)	2,936 (838)	4,144 (984)	3,651 (995)	4,449 (993)	2,920 (862)	3,494 (983)	3,409 (871)	4,291 (979)
FEV_1/FVC (%)	74.4 (8.1)	73.7 (8.3)	73.0 (8.1)	69.6 (9.7)	74.3 (7.3)	72.0 (7.8)	76.3 (6.6)	75.4 (9.5)	73.3 (8.7)	79.7 (7.0)	77.9 (6.3)
ALA (% of total FAs)	0.23 (0.09)	0.17 (0.06)	0.14 (0.04)	0.15 (0.06)	0.14 (0.05)	0.18 (0.11)	0.17 (0.07)	0.16 (0.06)	0.18 (0.10)	0.17 (0.08)	0.19 (0.08)
EPA (% of total FAs)	2.87 (1.66)	0.85 (0.57)	0.61 (0.36)	0.60 (0.39)	0.56 (0.25)	0.74 (0.48)	0.67 (0.42)	0.91 (0.72)	0.93 (0.73)	0.68 (0.42)	0.84 (0.61)
DPA (% of total FAs)	1.18 (0.21)	0.94 (0.17)	0.86 (0.20)	0.83 (0.17)	0.90 (0.17)	2.76 (0.46)	2.55 (0.45)	0.95 (0.23)	0.93 (0.22)	0.93 (0.21)	0.94 (0.21)
DHA (% of total FAs)	6.33 (1.52)	3.52 (0.93)	3.55 (0.98)	2.98 (0.95)	2.81 (0.86)	4.87 (1.37)	4.19 (1.22)	4.28 (1.37)	3.53 (1.33)	3.29 (0.99)	3.10 (1.12)
Total *n*-3 PUFAs (% of total FA)‡‡	10.60 (3.08)	5.48 (1.38)	5.16 (1.31)	4.56 (1.27)	4.41 (1.04)	8.56 (2.00)	7.59 (1.78)	6.29 (2.07)	5.57 (2.02)	5.07 (1.32)	5.08 (1.65)
Time difference between PFT and *n*-3 PUFAs, d§§	1 (6)	1705 (178)	353 (27)	362 (29)	0 (0)	3 (77)	0 (0)	1724 (116)	1769 (110)	0 (0)	0 (0)

Definition of abbreviations: AA = African ancestry; AGES = Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ALA = α-linolenic acid; ARIC = Atherosclerosis Risk in Communities; CARDIA = Coronary Artery Risk Development in Young Adults; CHS = Cardiovascular Health Study; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EA = European ancestry; EPA = eicosapentaenoic acid; FA = fatty acid; FHS (Gen3) = Framingham Heart Study—Generation 3 Cohort; FHS (Offspring) = Framingham Heart Study—Offspring Cohort; MESA = Multi-Ethnic Study of Atherosclerosis; NA = not applicable; *n*-3 PUFA = omega-3 polyunsaturated fatty acid; PFT = pulmonary function test; RS = Rotterdam (Netherlands) Study. Data are presented as mean (SD) unless otherwise indicated. Seven cohorts are included in total. All the cohorts measured *n*-3 PUFAs in plasma, except FHS, which measured *n*-3 PUFAs in red blood cells in its two subcohorts (the Offspring Cohort and the Generation 3 Cohort). The descriptive statistics presented are based on all participants in the main *n*-3 PUFA–PFT association analyses.

*In CHS, six EA participants and six AA participants were excluded as residual outliers (Studentized residual >4 for EA participants, and >3 for AA participants), based on the main effect model without the *n*-3 PUFA term. All other cohorts have descriptive statistics on participants before applying the exclusion of residual outliers. In addition, in CHS, 1,684 EA participants and 242 AA participants were used for the descriptive statistics of weight and FVC.

†The total sample size for the meta-analysis of omega-3 fatty acid biomarker associations with PFTs was 16,134, in which 13,629 were EA participants and 2,505 were AA participants.

‡The total sample size for the genome-wide interaction analyses of omega-3 fatty acid biomarkers (84.6% of those included in the *n*-3 PUFA–PFT association analysis) was 13,649, in which 12,306 were EA participants (90.3% of all EA participants) and 1,343 were AA participants (53.6% of all AA participants). Any discrepancy in numbers of participants for the *n*-3 PUFA–PFT association and discovery-phase genome-wide analyses was due to participants whose genetic data did not pass quality control.

§Genome-wide interaction analysis was not performed in these cohorts/subcohorts owing to their small sample sizes ($N < 500$).

‖The genome-wide interaction analysis in FHS was performed using two subcohorts combined, with adjustment of family relatedness.

¶CARDIA was used as a replication cohort to test novel associations from the genome-wide interaction analyses. The *n*-3 PUFA–PFT association analysis did not exclude participants according to their availability of genetic data. Therefore, the sample size was smaller for the genetic analysis.

**Number of participants with weight data was slightly different from the number of participants shown in this table for some cohorts. In CHS, 242 out of 243 AA participants and 1,687 out of 1,690 EA participants had weight data. In CARDIA, all 1,759 EA participants and 1,456 out of 1,461 AA participants had weight data.

††Descriptive statistics of pack-years was conducted among ever smokers.

‡‡Total *n*-3 PUFA biomarkers is the sum of ALA, EPA, DPA, and DHA in plasma or red blood cells.

§§The time difference refers to the interval between measurement of PFT and *n*-3 PUFA biomarkers. The difference is positive when the *n*-3 PUFA biomarkers were measured before the PFTs, whereas the value is negative when the *n*-3 PUFA biomarkers were measured after the PFTs. In MESA, 16 EA participants and 18 AA participants had missing data for the time difference variable.

Two novel loci were identified at cross-ancestry meta-analysis $P_{2df} < 5 \times 10^{-8}$ (Figures E6 and E7). For FEV_1, rs79992631, a downstream *C8orf4* SNP on chromosome 8p11, was identified when accounting for DPA interaction. However, because the signal was driven by a single cohort with suboptimal imputation quality at this SNP ($R^2 = 0.65$) and was not supported by other cohorts in the discovery meta-analysis with better imputation quality at this SNP, rs79992631 was not tested further, as it is likely a false positive. For FVC, rs11693320, a dipeptidyl peptidase–like 10 (*DPP10*) intronic SNP on chromosome 2q14 (Figure 3), was identified when accounting for DHA interaction. Meta-analysis across all discovery cohorts revealed that rs11693320 was associated with FVC at $P_{2df} = 4.5 \times 10^{-8}$ (Table 2). The rs11693320–FVC association was tested for replication in CARDIA (1,141 EA participants, 546 AA participants; Table 1) and found to be associated at $P_{2df} = 0.045$ with consistent directions of association (Table 2), and an overall $P_{2df} = 9.4 \times 10^{-9}$ across all cohorts, which passed a stringent Bonferroni-corrected cutoff of 1.25×10^{-8} that takes into account all four genome-wide interaction models (DHA and DPA with FEV_1 and FVC). The rs11693320-A allele, which has a similar frequency across ancestries (81% in EA participants, 79% in AA participants), was associated with reduced FVC ($\beta_{SNP} = -161.0$ ml, $P_{SNP} = 2.1 \times 10^{-9}$); this association was attenuated by higher DHA levels ($\beta_{SNP \times DHA\ interaction} = +36.2$ ml per 1% DHA of total FAs, $P_{SNP \times DHA\ interaction} = 2.1 \times 10^{-7}$). In the discovery cohorts, the rs11693320 and rs11693320 × DHA interaction effect sizes were larger in AAs ($\beta_{SNP} = -186.4$ and $\beta_{SNP \times DHA\ interaction} = 39.7$ in the single AA cohort compared with $\beta_{SNP} = -155.8$ and $\beta_{SNP \times DHA\ interaction} = 34.0$ in the EA-specific meta-analysis; Table 2). The same pattern was observed between AA and EA participants in the replication cohort (Table 2). Although not passing genome-wide significance, consistent directions were observed for the association of rs11693320 and its interaction with DHA on FEV_1 (meta-analysis β_{SNP} [SE] = −95.2 [23.9], $\beta_{SNP \times DHA\ interaction}$ [SE] = 19.9 [6.0], and $P_{2df} = 2.1 \times 10^{-4}$).

We also used our genome-wide results to look up previous GWAS-identified SNPs associated with *n*-3 PUFA phenotypes and PFTs. Results are shown in the online supplement and Tables E10 and E11.

DPP10 SNP Interaction with *n*-3 PUFA Biomarkers by Smoking Status and Sex

The joint 2*df* meta-analyses accounting for rs11693320 × DHA interaction on FVC was further explored in models stratified by smoking status, which suggested that the interaction was mainly driven by former smokers ($N = 5{,}373$; $\beta_{SNP} = -218.5$ ml, $P_{SNP} = 8.3 \times 10^{-6}$; $\beta_{SNP \times DHA\ interaction} = +53.8$ ml, $P_{SNP \times DHA\ interaction} = 6.7 \times 10^{-6}$). The directions of association were consistent in current and never smokers, but with weaker statistical evidence (current smokers: $N = 3{,}944$; $\beta_{SNP} = -130.4$ ml, $P_{SNP} = 0.15$; $\beta_{SNP \times DHA\ interaction} = +21.8$ ml, $P_{SNP \times DHA\ interaction} = 0.45$; and never smokers: $N = 4{,}332$; $\beta_{SNP} = -93.7$ ml, $P_{SNP} = 0.030$; $\beta_{SNP \times DHA\ interaction} = +16.4$ ml, $P_{SNP \times DHA\ interaction} = 0.13$).

When stratified by sex, the rs11693320 × DHA interaction finding on FVC was mainly driven by males ($N = 6{,}231$; $\beta_{SNP} = -223.0$ ml, $P_{SNP} = 2.5 \times 10^{-5}$; $\beta_{SNP \times DHA\ interaction} = +55.8$ ml, $P_{SNP \times DHA\ interaction} = 6.1 \times 10^{-5}$). The directions of association were consistent in females, but with weaker statistical evidence ($N = 7{,}418$; $\beta_{SNP} = -60.3$ ml, $P_{SNP} = 0.09$; $\beta_{SNP \times DHA\ interaction} = +11.6$ ml, $P_{SNP \times DHA\ interaction} = 0.20$).

Follow-up Bioinformatics Analysis

According to HaploReg v4.1 (35), three variants in high linkage disequilibrium ($r^2 > 0.8$) with rs11693320 are located within putative enhancer elements in lung tissue. Functional annotations of rs11693320 and variants with $r^2 > 0.8$ (1000 Genomes EUR) are provided (Table E12). Rs11693320 is a putative expression quantitative trait locus for *DPP10* in GTEx lung tissue, with its A allele being associated with lower expression ($P = 0.036$, $N = 383$) (37). To better characterize *DPP10* gene function, we used GeneMANIA to create a network of genes biologically related to *DPP10* (Figures 4 and E9). Within this network of 20 genes, 5 genes were coexpressed ($P < 0.05$) with *DPP10* in GTEx lung tissue: *DPP4*, *FMN2*, *FABP4,* and *VAT1L* were positively associated with *DPP10* expression, whereas *ADAM20* was inversely associated.

Discussion

Our study tested the associations of *n*-3 PUFA biomarkers with PFTs combining data across multiple cohorts, which showed positive associations of DHA and DPA with FEV_1 and FVC. The FEV_1 outcome had slightly larger magnitudes of association with *n*-3 PUFAs, consistent with the pattern observed in the only previous study that investigated plasma *n*-3 PUFA associations with PFTs in adults (22). Importantly, we found, to our knowledge for the first time, that the association of FEV_1 with DHA differed by smoking status ($P_{DHA \times smoking\ interaction} = 0.02$), with the magnitude of the association for current smokers ($\beta = 39$ ml per 1% [∼1 SD] higher DHA) being about threefold larger than the association in never ($\beta = 11$ ml) and former ($\beta = 13$ ml) smokers. We also found a significant interaction of DHA with current smoking on FEV_1/FVC, although the magnitude of the association was negligible (<1% increase per 1% higher DHA). In addition, we found a DPA × sex interaction such that the magnitude of the DPA association with FVC was larger in males than in females (larger by 10 ml per 1 SD [0.2%] higher DPA).

In genome-wide interaction meta-analyses, we identified the *DPP10* SNP rs11693320-A allele for its novel association with FVC ($\beta_{SNP} = -161.0$ ml), which was attenuated by higher DHA levels ($\beta_{interaction} = +36.2$ ml) (Table 2). To put the magnitude of the *DPP10*–FVC association into context, rs11693320-A was associated with 88.6-ml lower FVC at DHA level = 2% of total FAs (∼1 SD below the average DHA level), whereas rs11693320-A was associated with 16.2-ml lower FVC at DHA level = 4% of total FAs (slightly above the average DHA level). In comparison, 1 year of age-related FVC decline is ∼30 ml in US adults from the general population (39). Our findings indicate that nutrient biomarker levels might influence genetic factors underlying pulmonary function.

The only prior study to directly investigate *n*-3 PUFA biomarkers and PFTs in adults ($N = 593$) reported suggestive positive associations of DHA with percentage predicted FEV_1 and FVC, and a positive association of DPA with percentage predicted FVC in men only (22). Our study included large numbers, tested smoking interactions for the first time, and found a

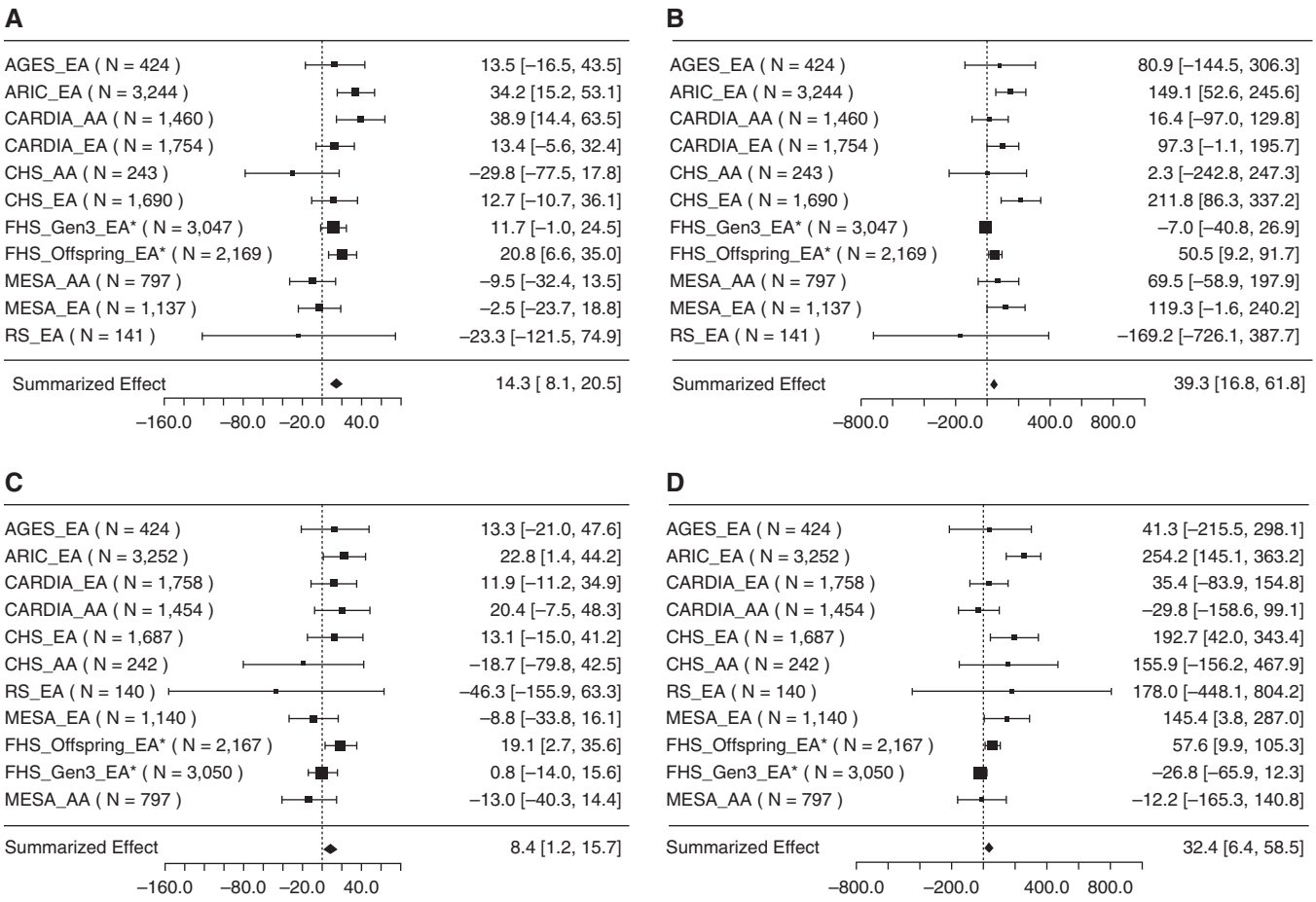

Figure 1. Forest plots of the meta-analysis of omega-3 fatty acid biomarkers on FEV_1 and FVC. Associations are presented for (*A*) docosahexaenoic acid (DHA)–FEV_1, (*B*) docosapentaenoic acid (DPA)–FEV_1, (*C*) DHA–FVC, and (*D*) DPA–FVC. β (ml) denotes the coefficient from the fixed-effects meta-analysis for each omega-3 fatty acid biomarker (DHA or DPA) on the pulmonary function outcome per 1% (of total fatty acids) increment, with its 95% confidence interval. The linear model in each cohort was adjusted for smoking status (never/former/current smokers), pack-years, sex, age, age^2, height, height^2, weight (FVC outcome only), and study center (when applicable). The vertical line in the center indicates no association of the omega-3 fatty acid biomarker with the pulmonary function outcome; β value to the right of the line indicates a positive effect, whereas β value to the left of the line denotes a negative or inverse effect. The size of the solid square for each cohort represents the variance of the β coefficient, so that cohorts with smaller variances have larger solid squares. Sample size of each cohort is shown in parenthesis. *FHS has omega-3 fatty acid biomarkers measured in red blood cells, rather than plasma. AA = African ancestry; AGES = Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC = Atherosclerosis Risk in Communities; CARDIA = Coronary Artery Risk Development in Young Adults; CHS = Cardiovascular Health Study; EA = European ancestry; FHS (Gen3) = Framingham Heart Study—Generation 3 Cohort; FHS (Offspring) = Framingham Heart Study—Offspring Cohort; MESA = Multi-Ethnic Study of Atherosclerosis; RS = Rotterdam (Netherlands) Study.

larger magnitude of the DHA association with FEV_1 in current smokers compared with former and never smokers. The positive associations of DHA and DPA with PFTs are biologically plausible and may be mediated by metabolic derivatives such as resolvins and protectins, which regulate the resolution of inflammation via mechanisms including the inhibition of proinflammatory gene expression and the clearance of inflammatory cells by macrophages (17). DHA-derived Resolvin D1 was shown to have anti-inflammatory effects in mice and human cell lines with cigarette smoke exposure (40).

The rs11693320 association with FVC was evident only by considering interaction with DHA. Rs11693320 did not attain genome-wide significance in standard 1*df* meta-analysis without DHA interaction in the model ($P = 1.7 \times 10^{-4}$; Table 2). Similarly, rs11693320 was not identified in previous GWASs of PFTs (6–12, 41). Our top *DPP10* SNPs, some of which were available as HapMap-imputed SNPs in a previous GWAS of FVC with a larger sample size ($N = 52{,}253$) (10), achieved only borderline nominal significance ($P = 0.06$–0.1) (Table E13). Moreover, rs11693320 had no association with DHA in our study ($P = 0.57$ across the five cohorts), and nearby HapMap-imputed *DPP10* SNPs also were not associated with the DHA phenotype in a CHARGE GWAS meta-analysis of plasma *n*-3 PUFAs (Table E13) (41).

DPP10 was previously identified as a candidate gene for asthma (42–45), and a single study of asthma candidate genes suggested that major alleles of *DPP10* SNPs were associated with both FEV_1 and FVC decline under a recessive mode of inheritance (46). Similarly, in our study, we found that the major allele of rs11693320 on *DPP10* was associated with lower FEV_1

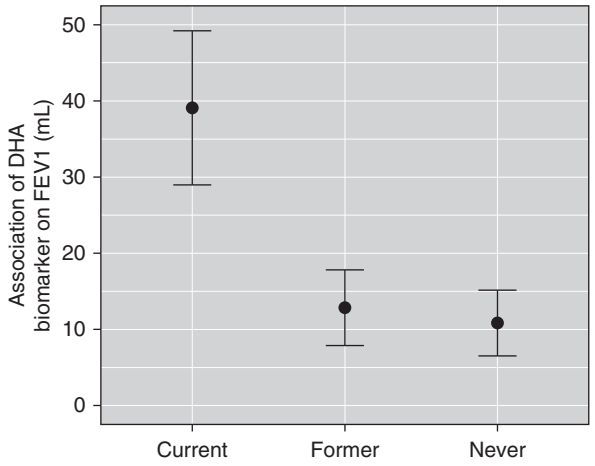

Figure 2. Meta-analysis of the association of DHA biomarker–FEV_1 outcome, by smoking status. Smoking status categories were never smoker, former smoker, and current smoker. The *y*-axis shows the coefficient β (unit: ml), which denotes that a 1% (of total fatty acids) higher DHA level was associated with a β ml higher FEV_1, in participants stratified by smoking status. The error bar represents ±1 SE. In total, 16,106 participants were used for the FEV_1 outcome. DHA = docosahexaenoic acid.

and FVC, although only the finding for FVC reached genome-wide significance. Prior GWAS for FVC similarly identified loci that were not detected in GWAS of FEV_1, suggesting that these correlated, but clinically different, measures have both shared and unique genetic risk factors (10). Previous speculation about the biological mechanism through which *DPP10* affects asthma relates to conduction of electric signals in the nervous system, which could affect the activity of airway smooth muscle (e.g., contraction) via neural regulation (44). *DPP10* is highly expressed in brain neurons (47) and slightly expressed in lung tissues (48). It encodes one member of the S9B family of serine proteases, which could be released to the extracellular space (49). The *DPP10*-encoded protein can bind to the voltage-gated potassium (K^+) channel and facilitate the trafficking of K^+ channel protein to the cell membrane (47).

Using the public bioinformatics tools GeneMANIA (38) and GTEx (37), we found five genes related to *DPP10* function and four of them positively coexpressed with *DPP10* in lung tissue. Based on previous evidence (49–53), only *FABP4* and *DPP4* play a role in pulmonary function and may potentially interact with DHA in this regard. The *FABP4* gene is a putative biomarker for systemic inflammation in patients with COPD, and FABP4 circulating level was associated with lower PFTs in patients with COPD (50) and nondiseased individuals (51). A small clinical trial ($N = 14$) reported that DHA + EPA supplementation, which increased serum DHA, led to a concurrent decrease in FABP4 level (52). The other *DPP10*-related gene, *DPP4*, plays a role in asthma pathogenesis, as *DPP10* does, albeit through a different mechanism (i.e., immunosuppression) (49). A study in diabetic patients directly linked *DPP4* to the DHA biomarker; the efficacy of a *DPP4* inhibitor on glycemic control was positively correlated with DHA nutriture ($r = 0.73$) (53). Thus, DHA may play a beneficial role in pulmonary function, potentially through influencing *DPP10*, *DPP4*, and *FABP4,* but further research is needed to investigate the interplay between these genes and DHA.

Given that *n*-3 PUFAs are postulated to mitigate inflammatory responses brought about by cigarette smoking, we performed smoking-stratified analyses and found that the rs11693320 effect size was largest in former smokers, when considering its interaction with DHA, suggesting potential effect moderation by cigarette smoking. A study of human fetal lung tissue reported that *in utero* smoking exposure was associated with methylation changes in *DPP10* (54). Our findings suggest an inverse association of *DPP10* SNPs with PFTs that are mitigated by circulating DHA levels, and the interplay among *DPP10*, DHA, and smoking status needs further investigation. We posit that current smoking, as compared with former smoking, induces additional perturbations to lipid homeostasis (e.g., lipid peroxidation of cell membranes of vulnerable cells such as airway epithelial cells expressing *DPP10*) via oxidative stress and epigenetic changes (including DNA methylation, histone modifications, and/or micro-RNA dysregulation), which might affect the beneficial effects of *n*-3 PUFAs and their attenuation of genetic risk factors on FVC. Moreover, the association pattern among former smokers may also underlie our observed sex-stratified results, whereby the largest effect sizes for rs11693320 and its interaction with DHA occurred in men, who are more likely to have smoked, smoked more heavily, and reported being more severely dependent on nicotine in their current and past smoking histories as compared with women (55, 56).

Our findings are likely to have strong external validity, and thus they are expected to generalize well to adult populations in the United States and Europe. Overall, average FEV_1/FVC of the included cohorts was in the expected 70–80% range for healthy US adults (Table E6), and the prevalence of COPD is expected to be similar to the US prevalence (~6.1%) (57). Participant selection bias is expected to be minimal given that all the measurements (spirometry, *n*-3 PUFA biomarkers, and genetic data) were collected either in all cohort participants or in a random set of participants. Finally, only 1,343 AAs contributed data for the genome-wide interaction analysis, which led us to combine EA and AA participants in cross-ancestry meta-analyses (total $N = 13{,}649$) to increase power. Even though the rs11693320 and rs11693320 × DHA interaction effect sizes were larger in AA than EA participants, which has been observed for other reported SNP associations with complex traits (e.g., *CHRNA5* SNP rs16969968 with cigarettes smoked per day) (58), drawing an inference of ancestral differences for this SNP was limited given fewer AA participants (total $N = 1{,}343$) than EA participants (total $N = 12{,}306$) available for study. Although the cross-sectional design prohibits direct causal inferences, these findings are strengthened by the internal consistency of findings across cohorts with different contexts. Future studies that investigate longitudinal PFTs and the complex interplay of fatty acid components are needed to

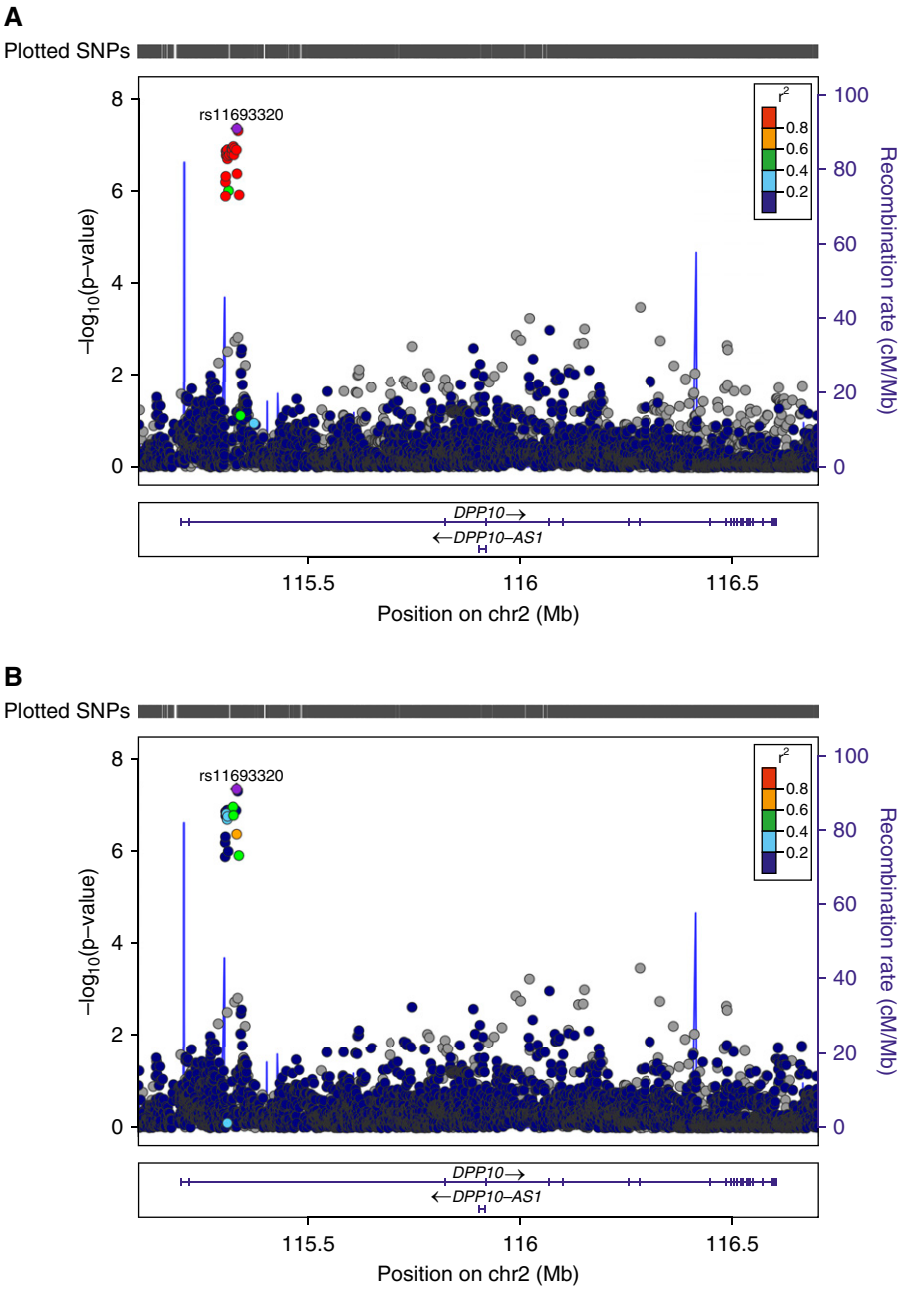

Figure 3. Novel *DPP10* locus identified at genome-wide significance ($P_{2df} < 5 \times 10^{-8}$) for FVC, accounting for SNP/indel × docosahexaenoic acid interaction. SNP/indel associations are shown from the cross-ancestry joint 2*df* meta-analysis across *DPP10* and its 100-kb flanking region (National Center for Biotechnology Information build 37 positions presented), using the LocusZoom tool. r^2 values between the top SNP rs11693320 and all other SNPs are shown in reference to the 1000 Genomes European (*A*) or African ancestry (*B*). Indels with missing r^2 values are indicated in gray. 2*df* = 2 degrees of freedom; indel = insertion/deletion.

further strengthen the causal inference of associations observed in our study.

This study has several strengths. First, we used objectively measured *n*-3 PUFA biomarkers, instead of self-reported dietary intake, as the exposures. The *n*-3 PUFA biomarkers reflect intake as well as interindividual differences in absorption and incorporation into phospholipids (for *n*-3 PUFAs from dietary sources) and metabolic efficiency (for *n*-3 PUFAs from endogenous biosynthesis). Therefore, it is a more reliable measure of *n*-3 PUFA nutrients that are available to tissues/organs, compared with self-reported dietary intake of *n*-3 PUFAs. Second, we conducted association analyses of *n*-3 PUFAs on PFTs across multiple cohorts that together had sufficient sample size to examine effect modification by smoking and by sex. Third, we investigated the genome-wide variant × nutrient interactions on PFTs via joint 2*df* meta-analyses and discovered a novel genetic association with pulmonary function, when considering the interaction with *n*-3 PUFAs, which was not identified previously using the standard GWAS approach with even larger sample sizes.

We found positive associations of DHA and DPA biomarkers with PFTs, specifically FEV_1 and FVC, and the magnitude of the DHA–FEV_1 association was about threefold larger in current smokers. This suggested a greater beneficial effect of *n*-3 PUFAs, especially DHA, on pulmonary function in current smokers. We also identified the *DPP10* locus, where the intronic rs11693320-A was inversely associated with FVC, and a higher DHA level attenuated this effect. Few genome-wide studies investigate how nutrient status and genetic predisposition can influence each other and affect PFTs, and the results of this study are important in contributing to the evidence base needed to provide targeted dietary advice for COPD prevention. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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Table 2. Cohort-Specific Results for the Association of *DPP10* rs11693320-A with FVC, with and without DHA Interaction Included in the Model

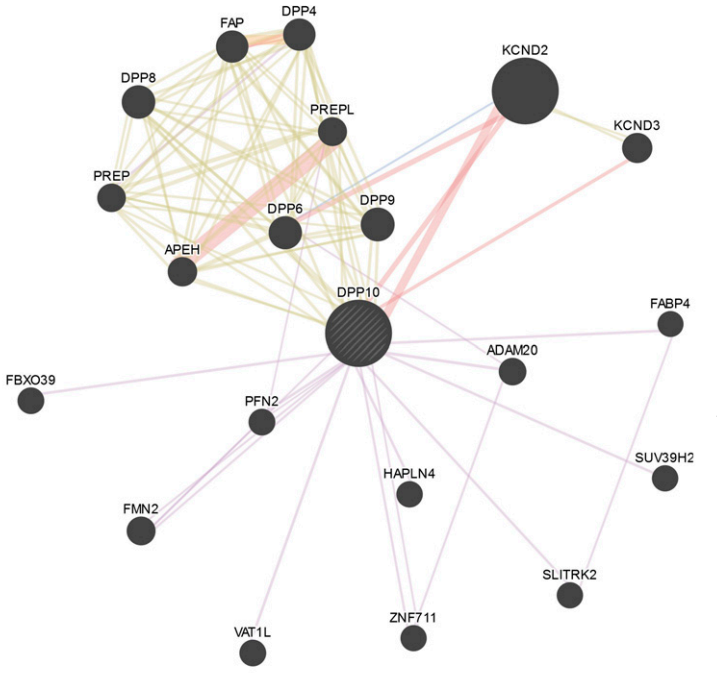
Cohort and Ancestry (Sample Size)	A Allele Frequency	Model without Interaction with DHA (*Total N = 13,649*)*		Model with Interaction with DHA (*Total N = 13,649*)†				
		β (SE)	*P*	β_{SNP} (SE)	P_{SNP}	$\beta_{SNP\times DHA\ interaction}$ (SE)	$P_{SNP\times DHA\ interaction}$	P_{2df}
Discovery cohorts								
ARIC EA (*n* = 3,143)	0.80	−65.9 (18.0)	2.4×10^{-4}	−145.6 (60.0)	0.015	28.3 (20.4)	0.17	4.4×10^{-4}
CHS EA (*n* = 1,684)	0.80	−72.6 (26.4)	0.0060	−15.0 (90.6)	0.87	−19.8 (29.1)	0.50	0.011
FHS EA (*n* = 5,198)	0.82	−8.6 (15.2)	0.57	−147.6 (56.9)	0.0095	34.7 (11.8)	0.0032	0.011
MESA EA (*n* = 1,137)	0.81	−15.8 (29.6)	0.30	−156.1 (84.9)	0.066	38.7 (22.2)	0.081	0.15
MESA AA (*n* = 797)	0.79	−16.0 (35.1)	0.20	−186.4 (116.6)	0.11	39.7 (25.1)	0.11	0.27
Discovery cohort meta-analysis (EA only)		−36.0 (10.0)	2.7×10^{-4}	−155.8 (28.7)	5.8×10^{-8}	34.0 (7.6)	7.4×10^{-6}	1.5×10^{-7}
Discovery cohort meta-analysis (EA and AA)		−34.9 (9.6)	2.9×10^{-4}	−157.1 (27.7)	$\mathbf{1.4 \times 10^{-8}}$	34.3 (7.2)	1.7×10^{-6}	$\mathbf{4.5 \times 10^{-8}}$
Replication cohort								
CARDIA EA (*n* = 1,141)	0.81	−49.6 (63.2)	0.43	−115.5 (160.1)	0.47	36.6 (47.3)	0.44	0.76
CARDIA AA (*n* = 546)	0.77	−32.4 (50.5)	0.52	−320.7 (153.1)	0.036	107.3 (43.1)	0.013	0.061
Replication cohort meta-analysis		−39.1 (39.4)	0.32	−222.6 (110.7)	**0.044**	75.2 (31.9)	**0.018**	**0.045**
Overall meta-analysis		−35.1 (9.3)	1.7×10^{-4}	−161.0 (26.9)	$\mathbf{2.1 \times 10^{-9}}$	36.2 (7.0)	2.1×10^{-7}	$\mathbf{9.4 \times 10^{-9}}$

Definition of abbreviations: 2*df* = 2 degrees of freedom; AA = African ancestry; ARIC = Atherosclerosis Risk in Communities; CARDIA = Coronary Artery Risk Development in Young Adults; CHS = Cardiovascular Health Study; DHA = docosahexaenoic acid; EA = European ancestry; FHS = Framingham Heart Study; indel = insertion/deletion; MESA = Multi-Ethnic Study of Atherosclerosis.

Genome-wide significant ($P < 5 \times 10^{-8}$) results and significant replication results ($P < 0.05$) are shown in bold. The imputation quality of rs11693320 ranged from 0.72 to 0.85 across discovery cohorts.

*Model included SNP/indel additive dosage as the predictor and age, age^2, sex, standing height, standing height^2, weight, study site (when applicable), current/former smoking (dummy variables, never smokers as the reference group), pack-years, and principal components as covariates.

†Model included DHA as the exposure, SNP/indel additive dosage, and SNP/indel interaction term with DHA as predictors, and age, age^2, sex, standing height, standing height^2, weight, study site (when applicable), current/former smoking (dummy variables, never smokers as the reference group), pack-years, and principal components as covariates.



Gene Symbol	Coefficient Estimate	Standard Error	P-value
DPP4	0.25992	0.04937	2.4×10^{-7}
FMN2	0.15635	0.0504	0.0021
FABP4	0.139496	0.050099	0.0056
ADAM20	–0.11103	0.05034	0.028
VATIL	0.10216	0.0509	0.046

Figure 4. GeneMANIA network built around *DPP10*. Twenty genes are included in this network. Five of the network genes were coexpressed with *DPP10* in GTEx v7 lung tissue ($N = 383$), after adjustment of sex, age, and three genotyping principal components. The associations (coefficient estimate, SE, and *P* value) with *DPP10* expression in GTEx lung tissue are shown in the table portion of the figure (all $P < 0.05$).

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Supplemental Methods

Cohorts and Participants

Seven cohorts in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium were used for the meta-analysis of omega-3 poly-unsaturated fatty acid (n-3 PUFA) biomarkers on pulmonary function, with a total sample size of 16,134 participants. Among these cohorts, the Framingham Heart Study (FHS) is sub-divided into the Offspring cohort and the Generation 3 cohort (Gen3). One cohort [Multi-Ethnic Study of Atherosclerosis (MESA)] includes Hispanic and Chinese participants, but these were not included in this meta-analysis study given the limited sample size.

Our genome-wide interaction analyses focused on the five largest cohorts (N>500): ARIC EAs, CHS EAs, FHS EAs, and MESA EAs and AAs for discovery, and CARDIA EAs and AAs for replication. Besides having limited statistical power, small sample sizes can produce inflated type-I error, especially when analyzing interactions (1, 2). We found genomic control λ (λ_{gc}) values ranging from 1.14 to 1.33 for the smallest cohorts; $\lambda_{gc}=1$ is optimal, whereas $\lambda_{gc}>1.1$ is viewed as problematic in the conduct of genome-wide meta-analysis (3). All cohorts with N>500 had λ_{gc} values ranging from 0.98 to 1.07. Other genome-wide interaction studies conducted in the CHARGE consortium had similar minimum cohort-specific sample sizes (4-7).

An analysis pipeline was developed and distributed to each cohort, in which we harmonized the definitions and units of outcomes, exposures, and other covariates, as well as the statistical models. The exclusion criteria for the analyses of n-3 PUFA biomarkers (**Table E1**) included missing data on pulmonary function, unacceptable pulmonary function data that failed to meet the criteria of American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines (**Table E4** for more details

about the ATS/ERS criteria used in each cohort), and missing data on n-3 PUFAs and/or other covariates.

Genotyping, Quality Control, and Imputation

Participants were excluded from the genome-wide interaction analyses if they had poor call rate, unusually high heterozygosity, outlying ancestry, and/or relatedness (for population-based studies only) (see details in **Table E2**). Relatedness was not used as an exclusion criterion for FHS; instead, relatedness was taken into account in statistical analyses of this family-based study.

Different genotyping platforms were used across the cohorts. Genotyped single nucleotide polymorphisms (SNPs) were excluded if they had poor call rate, Hardy Weinberg disequilibrium, high duplicate discordance rates, and/or monomorphism (**Table E3**). Imputation was performed using 1000 Genomes reference panels (8) to harmonize SNPs and insertions/deletions (indels) for the meta-analysis (**Table E3**). Cohort-specific genomic data are available on the database of Genotypes and Phenotypes (dbGaP) via accession numbers: ARIC (phs000280), CARDIA (phs000285), CHS (phs000287), FHS (phs000007), and MESA (phs000209).

Pulmonary Function Test (PFT) Outcomes

Spirometry testing was planned for all the participants who remained in the cohorts at the time of measurement (**Table E4** for measurement details in each cohort), except MESA and AGES (Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland), in which pulmonary function was measured in a random subset (~54% of the MESA population and ~78% of the AGES population, respectively) (**Table E1**). Cohorts adhered to strict quality control and American Thoracic Society/European Respiratory Society standardization guidelines once released. Forced expiratory volume in the first second (FEV_1) and its ratio with forced vital capacity (FVC) are commonly used parameters to assess airway obstruction, whereas

FVC is an indicator of restrictive lung disorders; across the PFT outcomes, lower values indicate worse pulmonary function. Pre-bronchodilator pulmonary function data was used, and results would likely have remained similar if post-bronchodilator data was used, given the expected small percentage of participants with evidence of asthma (9).

N-3 PUFA Biomarkers

Measurement of n-3 PUFA biomarkers was planned for either all the participants with blood samples available, or a random set of participants (**Table E1**). All cohorts have PFTs and n-3 PUFA biomarkers measured concurrently or within 1 year on average, with the exception of MESA and RS (Rotterdam Study), in which the PFTs were performed ~5 years after the n-3 PUFA biomarkers were measured (see **Table E5** for details of measurement time in each cohort). The representativeness of single n-3 PUFA measurement with long-term n-3 PUFA status is fairly strong, with correlations ranging from 0.5 to 0.8 for EPA, DPA, and DHA between measurements separated by 6 years in a subset of CHS participants (10) and modest correlations over a 15-year period (ranged from 0.2–0.5 for ALA, EPA, DPA, and DHA) (11). Therefore, a single measurement of the n-3 PUFA biomarker and a corresponding PFT measurement within 5 years is not likely to lead to severe bias in the estimated cross-sectional association of n-3 PUFA biomarkers with pulmonary function. In addition, the n-3 PUFAs have been reported to be stable for about 4 years in red blood cells (12), and over 10 years in plasma, if stored at -80℃ (13).

Statistical Analysis in Individual Cohorts

Using linear regression models, covariates measured concurrently with PFTs—smoking status (indicator variables for current and former smoking, with never smoking as the reference), pack-years (defined as number of packs of cigarettes smoked per day, multiplied by number of years the participant has smoked), sex, age

(unit: year), age^2, standing height (unit: meter), standing height^2, weight (unit: kilogram, for FVC outcome only), and study center (if applicable for studies with more than one site)—were adjusted to reduce potential confounding. Age^2 and height^2 were included to more fully adjust for the non-linear relationship of age and height to PFTs (14), as done in prior GWAS (9, 15, 16). CHS used covariates measured concurrently with n-3 PUFA biomarkers, and they were within 1 year of the PFT measures (**Table E5**). The FHS models added a random effect to account for familial relatedness. Weight was included in the FVC analyses only because it could affect the expiratory reserve volume included in FVC, which is not relevant for FEV_1. In preliminary models without the n-3 PUFA predictor term included, participants who had studentized residuals greater than ±3 (or ±4), depending on each cohort, were excluded from further analyses. The number of participants excluded could vary across the three PFT outcomes (FEV_1, FVC, and FEV_1/FVC) (**Table E1**). After exclusion of outliers, ancestry-specific association testing of each n-3 PUFA with each PFT measure was conducted. Interactions of select n-3 PUFAs with smoking status and with sex on PFTs were also tested.

For the genome-wide testing of SNP/indel (henceforth, collectively referred to as SNP) and SNP × n-3 PUFA biomarker interaction, each cohort ran four genome-wide linear regression models that included FEV_1 or FVC as the outcome, DPA or DHA as the n-3 PUFA exposure, and SNP additive dosage and SNP × n-3 PUFA (DPA or DHA) interaction term as the predictors (linear regression model: FEV_1 or FVC ~ DHA [or DPA] + SNP + DHA [or DPA] × SNP + other covariates). The covariates are the same as the ones included for the associations of n-3 PUFA biomarkers on PFTs, with ancestral principal components (PCs) added to minimize confounding by population stratification. Models were run separately by cohort and ancestry using ProbAbel (17), R (18) or SUGEN (19) (**Table E3**). Genomic control

(gc) was applied to results by correcting the p-values via a genomic inflation factor (λ_{gc}). λ_{gc} was defined as the ratio of observed median chi-square statistics with 2 degree-of-freedom (2df) over the expected median, to quantify the extent of inflation, which could lead to false positive findings.

Meta-Analysis of the Associations of N-3 PUFAs and Interactions with Smoking on PFTs

Both ancestry-specific and cross-ancestry meta-analyses were performed. The interaction term of the n-3 PUFA (ALA, EPA, DPA, DHA) and smoking status (current smokers and former smokers, with never smokers as the reference) was meta-analyzed. If the interaction term was significant, additional meta-analysis was conducted stratified by smoking status. The cohort-specific n-3 PUFA-PFT associations by smoking status were calculated from the requested coefficients, standard errors, and covariance, with equations shown below.

$$\beta_{\text{n-3 PUFA in current smokers}} = \beta_{\text{n-3 PUFA in never smokers/reference}} + \beta_{\text{interaction of n-3 PUFA and current smokers}} \quad (1)$$

$$\text{Var}_{\text{n-3 PUFA in current smokers}} = \text{Var}_{\text{n-3 PUFA in never smokers/reference}}[a] + \text{Var}_{\text{interaction of n-3 PUFA and current smokers}}[b] + 2 \times \text{Cov}([a],[b]) \quad (2)$$

Fixed-effects meta-analysis was used to evaluate the magnitude and significance of the association of each n-3 PUFA biomarker with each PFT measure, while random-effects meta-analysis was used to assess the between-study heterogeneity. The metafor package (version 1.9-9) in R (version 3.3.1, R Foundation for Statistical Computing, Vienna, Austria) was used for the meta-analyses and follow-up meta-regression analyses of the n-3 PUFA-PFT associations, and the interaction meta-analyses of n-3 PUFA with smoking on PFT measures.

Joint 2df Meta-Analysis to Test Genome-Wide Interactions with N-3 PUFAs

Joint 2df testing is constrained on a joint null hypothesis for the variant main effect and interaction; the regression framework means that it can accommodate other

covariates and is amenable to various coding schemes for predictors and outcomes. Joint 2df testing has been shown repeatedly in theoretical (1, 20-22) and empirical (4, 23, 24) studies to offer more statistical power over a range of variant main and interactive effects, as compared to 1df tests of variant main or interactive effects only. In this study, ancestry-specific coefficients of the SNP additive dosage (β_{SNP}) and the SNP × n-3 PUFA interaction term ($\beta_{interaction}$) in each cohort were combined via joint 2df meta-analysis using METAL (25), with genomic control applied for variants with minor allele frequency (MAF) > 5% and imputation quality > 0.3. Meta-analysis results are presented for SNP variants tested in two or more cohorts.

Bioinformatics Analysis

HaploReg v4.1 (26) and Roadmap Epigenomics data [chromHMM (for chromatin state discovery and characterization)] (27) were used to functionally characterize the novel locus and any variants in high linkage disequilibrium (LD) ($r^2 \geq 0.8$ in 1000 Genomes Phase 1 EUR reference panel), focusing on predicted functional elements in lung tissue. The predicted fetal lung chromatin state based on Roadmap Epigenomics chromatin immunoprecipitation sequencing data (27) was viewed in the UCSC Genome Browser using the GRCh37/hg19 human assembly. Presence of certain chromatin marks could indicate the presence of an enhancer element. If a variant of interest is located nearby a region with predicted enhancer activity, it may be more likely to influence the expression of nearby (or even distant) genes. The Genotype-Tissue Expression Project (GTEx, version 7) *cis*-expression quantitative trait locus (*cis*-eQTL) results were used to look up whether the novel intronic variant is an eQTL for its host gene in lung tissue (28). GeneMANIA, a gene function prediction tool, was used to construct a network of genes biologically related to the gene of interest (29). R v3.2.4 was used to run linear regression models to test for association between network genes identified from GeneMANIA and the gene of

interest in lung tissue (linear regression model: normalized expression of gene of interest in GTEx ~ normalized expression of GeneMANIA network gene in GTEx + sex + age + genotyping principal components), using GTEx v7 RNA-Seq data (fully processed, filtered and normalized expression data, as previously described) (28). Nominal p-value of 0.05 was used as a significance threshold for co-expressions of genes in lung tissues.

Supplemental Results

N-3 PUFA Distributions

Of the n-3 PUFAs, DHA had the highest level (average of 3.7% of total FAs); ALA had the lowest level (average of 0.2% of total FAs; **Table E6**). ALA, EPA, and DHA levels were similar in plasma phospholipids and red blood cells. DPA measured in red blood cells was nearly three times more concentrated than in plasma (~2.6% vs ~0.9%, **Table E6).** Whether the differences between red blood cell and plasma measurements reflect true differences between cohorts or differences that derive mainly from compartment of measurement cannot be fully addressed by these data. Average n-3 PUFAs ranged from 4.4% of total FAs to 10.6% of total FAs across cohorts (**Figure E1**).

Meta-Analysis of N-3 PUFA Associations with PFTs

Cross-ancestry meta-analyses revealed positive associations of DHA and DPA with FEV_1 and FVC at $P<0.05$. These associations were largely driven by EA participants, which comprise most of the total sample size (**Table E8** for ancestry-specific results). To explore the linearity of the n-3 PUFA-PFT associations across cohorts, a meta-regression analysis tested whether the β coefficients varied by mean of each n-3 PUFA; the cohort-specific β and mean n-3 PUFA level had little to no association (results not shown), supporting a linear n-3 PUFA-PFT association across

the range of each n-3 PUFA. Also, sensitivity analyses demonstrated that DHA and DPA were robustly associated with FEV_1 and FVC, when limited to cohorts with n-3 PUFAs measured in plasma only (**Figure E2)** or cohorts with n-3 PUFA measurements within one year of PFTs (**Figure E3**).

Targeted Look-up of Established N-3 PUFA and PFT-Related Variants

We used our 2df meta-analysis results to look-up previous GWAS-identified SNPs associated with n-3 PUFA phenotypes (**Table E10**). Using Bonferroni correction for 14 variants ($P<3.6\times10^{-3}$), one previously identified DPA-related SNP, rs174468 (upstream of *FADS3* on chromosome 11), was associated with FEV_1 when considering its interaction with DPA ($P_{2df}=1.8\times10^{-3}$).

Similarly, we used our 2df meta-analysis results to look-up SNPs identified in previous GWAS of PFTs (**Table E11**). Using Bonferroni correction for 199 variants ($P<2.5\times10^{-4}$), ten previously identified FEV_1-related SNPs were associated with FEV_1 in this study when considering interaction with either DHA or DPA. However, signals were primarily driven by main effects, not interactive effects with n-3 PUFAs ($P_{SNP\times n\text{-}3\ PUFA\ interaction}=0.11–1.00$).

Table E1. Flowchart of sample size dynamics in each cohort for the meta-analysis of omega-3 fatty acid biomarker associations with pulmonary function tests, stratified by ancestry[*]

European Ancestry Cohort	**CARDIA[§]**	**CHS[ll]**	**MESA[**]**	**AGES[††]**	**ARIC[§§]**	**FHS[lll]**	**RS[***]**
Original sample size	2,478	4,346	2,501	5,519	11,478	6,158	9,895
Excluded for the following reasons:							
Missing PFT (outcome of interest)	-632	-980	-1,119	-2,672	-47	-354	-2,542
Unacceptable PFT (outcome of interest)	0	-415	0	-16	-9	NA	-2,774
Missing height	0	-43	0	0	0	0	-1
Missing gender	0	0	0	0	0	0	0
Missing age	0	0	0	0	0	0	0
Missing smoking status	-15	-63	-12	-63	-7	-123	-6
Missing pack-years	0	-71	-51	-27	-130	-116	-172
Missing site (if applicable)	0	0	0	0	0	0	0
Missing genetic data[†]	0[†††]	-786	0	-1,056	-1,947	-344	-590
Missing n-3 PUFA biomarkers data (exposure of interest)	-72	-292	-170	-1,261	-6,084	0	-3,669
Missing weight (for the FVC analysis only)	0	-3	0	0	0	0	0
Sample size for the FEV_1 analysis[‡]	***1,754***	***1,690***	***1,137***	***424***	***3,244***	***5,216***	***141***
Sample size for the FVC analysis[‡]	***1,758***	***1,687***	***1,140***	***424***	***3,252***	***5,217***	***140***
Sample size for the FEV_1/FVC analysis[‡]	***1,753***	***1,690***	***1,134***	***424***[‡‡]	***3,233***	***5,205***	***141***
African Ancestry Cohort	**CARDIA[§]**	**CHS[ll]**	**MESA[**]**				
Original sample size	2,637	885	2,575				
Excluded for the following reasons:							
Missing PFT (outcome of interest)	-1,053	-262	-1,646				
Unacceptable PFT (outcome of interest)	0	-122	0				
Missing height	-5	-3	0				
Missing gender	0	0	0				
Missing age	0	0	0				
Missing smoking status	-12	-5	-17				
Missing pack-years	0	-22	-37				
Missing site (if applicable)	0	0	0				
Missing genetic data[†]	0[†††]	-49	0				
Missing n-3 PUFA biomarkers data (exposure of interest)	-106	-173	-65				
Missing weight (for the FVC analysis only)	-5	-1	0				
Sample size for the FEV_1 analysis[‡]	***1,460***	***243***	***797***				
Sample size for the FVC analysis[‡]	***1,454***	***242***	***797***				
Sample size for the FEV_1/FVC analysis[‡]	***1,440***	***243***	***797***				

Abbreviations: AGES = Age, Gene, Environment, Susceptibility Study – Reykjavik; ARIC = Atherosclerosis Risk in Communities; CARDIA = Coronary Artery Risk Development in Young Adults; CHS = Cardiovascular Health Study; FEV_1 = Forced expiratory volume in the first second; FHS = Framingham Heart Study; FVC = Forced vital capacity; MESA = Multi-Ethnic Study of Atherosclerosis; n-3 PUFA = Omega-3 poly-unsaturated fatty acid; PFT = Pulmonary function test; RS = Rotterdam Study.

[*]The final sample size of each cohort for each outcome variable is shown in the last three rows, stratified by ancestry.

[†]Participants who did not have genetic data were excluded for consistency and comparison with the genome-wide analyses.

[‡]For each outcome, participants whose studentized residual absolute value is greater than 3 (or 4), depending on each cohort, were excluded.

[§]PFTs were measured in year 20, therefore some CARDIA participants might have dropped out at that time point.

[ll]In CHS, PFTs were measured in year 6, therefore some participants might have dropped out at that time point. In addition, only those who did not have cardiovascular diseases at baseline, had available DNA, and consented to genetic testing had genetic data available (N= 3,865 out of 5,231). In terms of n-3 PUFA biomarker data, they were measured in all available blood samples in year 5 (N= 3,941).

[**]PFTs were measured in 3,965 participants who were enrolled in the MESA Lung Study (30, 31). The MESA Lung Study enrolled 3,965 participants out of 4,484 selected who were randomly sampled among those who consented to genetic analyses, underwent baseline endothelial function measures, and attended an examination in the MESA-Lung recruitment period in 2004-2006 (99%, 89%, and 91% of the MESA cohort, respectively) (31). In addition, the final sample size for each outcome additionally excluded participants who were related genetically ($N_{\text{European Ancestry}}$=9, $N_{\text{African Ancestry}}$=9).

[††]In AGES, only a random set of participants had PFTs (N= 3,000 out of 5,519) (32), and n-3 PUFA biomarkers (N = 1,012 out of 5,519) measured (33). In addition, only 3,219 out of 5,519 participants had genetic data (34).

[‡‡]In AGES, for FEV_1/FVC, two participants had residuals around 4.3 but were not filtered out. We would not expect this to influence the results much given that this was such a small number, and the residual values were close to 4.

[§§]In ARIC, genotyping was planned for the whole cohort (34). N-3 PUFA biomarkers were measured only in the Minneapolis study center, out of the 4 study centers across U.S (N=4,009) (35).

[lll]The flowchart of sample size in FHS has combined participants in the Offspring cohort and the Generation 3 cohort. FHS only has participants with acceptable PFT measures, therefore the exclusion criteria of unacceptable PFTs are not applicable here. N-3 PUFA biomarkers were measured at Exam 8 of the Offspring cohort, and at Exam 2 of the 3rd Generation cohort. The starting sample size for Exam 8 of the Offspring cohort and Exam 2 of the 3rd Generation cohort was 6,158.

[***]In RS, spirometry was not carried out in the cohort until 2002. At that time point, the total sample size of participants who still visited the research center was 9,895. Plasma n-3 PUFAs were measured for a nested case-control study of depression in the RS-I cohort during Exam 3 and only the participants in the control group were included in this meta-analysis. They were randomly selected from the RS-I cohort among those who had negative results for the depression screening (N = 461) (36).

[†††]In CARDIA, which was used for replication of top findings from the genome-wide interaction analyses, the analysis of omega-3 fatty acid biomarkers with pulmonary function did not exclude participants according to the availability of genetic data.

Table E2. Genotyping QC for participants and genetic analysis sample size in each cohort*

Study (ancestry)	QC filters for excluding participants	N, genotyped participants passing QC	N, genotyped participants passing QC with PFT, n-3 FA biomarkers and complete covariate data
ARIC (EA)	call rate<95%, sex mismatch, discordances with prior genotyping, >8 SD for any of the first 10 principal components, outlying average identity-by-state estimates, or first-degree relatives	9,338	3,143
CHS (EA)	call rate<95%, non-European ancestry, sex mismatch, or discordance with prior genotyping	3,268	1,684
FHS – Offspring (EA)	call rate<97%, non-European ancestry heterozygosity>5 SD from the mean, or >1000 Mendelian errors	3,753	2,158
FHS – Gen3 (EA)	call rate<97%, non-European ancestry heterozygosity>5 SD from the mean, or >1000 Mendelian errors	3,893	3,040
MESA (EA)	call rate < 95%, unexpected first-degree relatives, or first three PCs of ancestry > 3.5 SD from the mean	2,685	1,137 for FEV_1 and 1,140 for FVC
MESA (AA)	call rate < 95%, unexpected first-degree relatives, or first three PCs of ancestry > 3.5 SD from the mean	2,588	797
CARDIA (EA)	call rate < 98%, non-European ancestry, sex mismatch, sample duplicates, or first- or second-degree relatives	1,663	1,141
CARDIA (AA)	call rate < 98%, low heterozygosity (inbreeding coefficient F < -0.15), sex mismatch, sample duplicates, or first- or second-degree relatives	955	546

Abbreviations: AA = African ancestry; ARIC = Atherosclerosis Risk in Communities; CARDIA = Coronary Artery Risk Development in Young Adults; CHS = Cardiovascular Health Study; EA = European ancestry; FHS (Offspring) = Framingham Heart Study—Offspring Cohort; FHS (Gen3) = Framingham Heart Study—Generation 3 Cohort; MESA = Multi-Ethnic Study of Atherosclerosis; n-3 FA = Omega-3 fatty acid; PFT = Pulmonary function test; QC = Quality control; SD = Standard deviation.

*ARIC, CHS, FHS, and MESA were used for discovery, and CARDIA was used for replication.

Table E3. Genotyping, QC, and analysis details for genotyped SNPs[*]

Study (ancestry)	Genotyping platform	QC filters for excluding genotyped SNPs	N, genotyped autosomal SNPs passing QC	Imputation software	1000 Genomes imputation reference panel, using all available individuals	N, imputed autosomal SNPs used for analysis	Statistical analysis software
ARIC (EA)	Affymetrix 6.0 chip freeze 3	call rate < 95%, HWE P < 10^{-6}, MAF < 0.001, or no chromosomal location	719,415	Michigan Imputation Server, using minimac	phase 3 (version 5)	15,482,662	SUGEN (19)
CHS (EA)	Illumina 370CNV merged with ITMAT-Broad-CARe Illumina iSELECT	call rate < 97%, no heterozygotes, HWE P < 10^{-5}, > 2 duplicate errors or, Mendelian inconsistency (for HapMap CEU trios), or SNPs not found in HapMap	359,592	MaCH to pre-phase, minimac	phase 1 (version 3)	6,375,477 (for FEV_1) 6,375,546 (for FVC)	R (18)
FHS - Offspring (EA)[†]	Affymetrix 500K + 50K Human Gene Focused Panel	call rate < 96.9%, HWE P < 10^{-6}, MAF < 1%, Mendelian errors > 1000,	412,053	MACH/minimac (37)	phase 1 (version 3)	6,194,157	R (18)
FHS – Gen3 (EA)[†]							

		not being on chromosomes 1–22 or X, duplicates					
MESA (EA)	Affymetrix 6.0	call rate < 95%, heterozygosity > 53%, or monomorphic SNPs	897,981	Minimac3	phase 3 (version 5)	6,779,137	ProbAbel (17)
MESA (AA)						9,020,042	
CARDIA (EA)	Affymetrix 6.0	call rate < 95%, HWE P < 10^{-5}, duplicates, monomorphic SNPs	610,015	BEAGLE (38)	phase 1 (version 3)	NA‡	ProbAbel (17)
CARDIA (AA)	Affymetrix 6.0	call rate < 95%, HWE P < 10^{-5}, duplicates, MAF < 1%	682,448	Minimac (39)	phase 1 (version 3)	NA‡	ProbAbel (17)

Abbreviations: AA = African ancestry; ARIC = Atherosclerosis Risk in Communities; CARDIA = Coronary Artery Risk Development in Young Adults; CHS = Cardiovascular Health Study; EA = European ancestry; FHS (Offspring) = Framingham Heart Study—Offspring Cohort; FHS (Gen3) = Framingham Heart Study—Generation 3 Cohort; HWE = Hardy Weinberg equilibrium; MAF = Minor allele frequency; MESA = Multi-Ethnic Study of Atherosclerosis; QC = Quality control; SNP = Single nucleotide polymorphism.

*ARIC, CHS, FHS, and MESA were used for discovery, and CARDIA was used for replication.

†To account for family relatedness, FHS used the linear regression models with a robust variance method via generalized estimating equations, in which each extended pedigree is one cluster and an independent working correlation structure is implemented.

‡Not applicable (NA) because although genome-wide imputed genotype data are available in CARDIA, this cohort was used for replication testing of the top finding in the current study.

Table E4. Details of spirometry and omega-3 fatty acid biomarkers in each cohort

Cohort	Cohort description	Spirometry	Omega-3 fatty acids biomarkers
AGES	The Age, Gene/Environment Susceptibility – Reykjavik Study (AGES) came from the Reykjavik study, a cohort initiated in 1967 and included a random sample of 30,795 men and women who were born in 1907-1935 and lived in the greater Reykjavik area of Iceland in 1967, and they were divided into 6 groups (40). The AGES examinations started in 2002 and 11,549 participants in the Reykjavik Study were still alive at that time. Recruitment into the AGES study was randomly selected within the 6 groups of participants. The AGES exams ended in 2006 with a sample size of 5,764 survivors (32, 41).	A Vitalograph Gold Standard Plus (Vitalograph Ltd., Buckingham, UK) was used to carry out spirometry through a disposable mouthpiece on participants who were in a sitting position. The spirometer was routinely calibrated with 1L syringe. The spirometry procedure was detailed by a technician before testing. The pulmonary function testing was deemed successful if there were at least two acceptable maneuvers, defined as no more than 300mL difference between the two attempts for at least 6 seconds in each blow. Pre-bronchodilation spirometry testing was conducted in only the first 2 years (41).	Fasting blood was collected and plasma was stored at -80℃. Fatty acids (FAs) in the phospholipid fraction (PL) were measured, which reflects the short-term dietary intake of FAs (weeks to months) and the pool of FAs available to tissues. The analyses of FAs were conducted at the Fred Hutchinson Cancer Research Center (Seattle, WA). PLs was separated from other lipids using thin layer chromatography (TLC). Fatty acid methyl esters were generated by transesterification and isolated by Agilent 7890 gas chromatograph (GC) with a flame ionization detector and a Supelco-fused silica 100-m capillary column SP-2560. FAs were expressed as a weight percentage %. The coefficients of variation (CV) of pooled quality-control samples for EPA and DHA were 2.05% and 1.44% (33).
ARIC	The Atherosclerosis Risk in Communities (ARIC) Study is a population-based cohort of about 16,000 middle-aged men and women recruited from 4 communities in U.S. in 1987-1989. They went through a 3 to 4-hour examination in clinics at baseline. For this study, only participants	A Collins Survey II water-sealed spirometer (Warren E. Collins Inc., Braintree, MA) was used to carry out spirometry at visits 1 and 2. SensorMedics model 1022 dry rolling seal spirometers (OMI, Houston, TX) were used to carry out spirometry at visit 5. The spirometer was calibrated daily, and a single pulmonary function reading center was	Fasting blood was collected. Plasma was stored at -70℃. One technician analyzed fatty acid composition about 2 years later. The cholesterol ester (CE) and PL were extracted with chloroform/methanol, and separated using TLC. The methyl esters of fatty acids in CE and PL were measured separately by a Model 5890 GC (Hewlett-Packard, Avondale, PA). A total of 28

	from the suburban Minneapolis area were included given the n-3 PUFA biomarker measures were only conducted in this population (35, 42).	used to standardize the spirometry testing across the four study sites in ARIC. The test was deemed successful if there were three acceptable attempts (43).	fatty acids were identified using GC. The short-term (usually several weeks) reliability coefficients were 0.31 for EPA in phospholipids and 0.58 for DHA in phospholipids. The long-term (within 3 years) reliability coefficient was 0.51 for EPA in phospholipids. Overall the reliability coefficient for DHA was greater than the one for EPA (42). The concentration was expressed as % of total fatty acids. The correlations between plasma and dietary polyunsaturated fatty acids were 0.25 for PL and 0.31 for CE (44).
CARDIA	The Coronary Artery Risk Development in Young Adults (CARDIA) study is a longitudinal cohort that recruited participants from 4 U.S. metropolitan areas, including Birmingham, AL, Chicago, IL, Minneapolis, MN, and Oakland, CA. CARDIA began in 1985-1986 and comprised 5,115 white and black young adults aged 18-30 yrs (45).	A dry rolling-sealed SensorMedics model 1022 OMI spirometer (Viasys, Yorba Linda, CA) was used at year 20, and the 2005 American Thoracic Society (ATS)/ European Respiratory Society (ERS) criteria was followed (46). The Pulmonary Waveform Generator (MH Custom Design and Manufacturing, Midvale, UT) validated the accuracy of the spirometer. The OMI spirometers performed better than the ATS criteria for accuracy and precision (47).	Fasting plasma samples were collected at Year 20 and EDTA plasma was frozen at -70℃. Lipids were extracted from plasma through chloroform/methanol. CE, PL, triglyceride, and free FAs were separated using TLC. The fatty acid methyl esters were generated from transesterification from the PL fraction and were measured by GC with a flame ionization detector. 28 fatty acids were identified and were expressed as % of total fatty acids (48).
CHS	The Cardiovascular Health Study (CHS) comprised 5,201 men and women aged ≥ 65 yrs who were recruited from 4 U.S. communities, including Forsyth County, NC, Sacramento County,	A water-sealed spirometer (Collins Survey, Collins Medical, Inc., Braintree, MA) and software from S&M instruments (50) were used to carry out pulmonary function testing at years 2, 6, and 9, with accuracy validated, according to	Fasting blood samples were collected and stored at -80℃. Fatty acids in plasma PL were measured at the Fred Hutchinson Cancer Research Center. A total of 45 fatty acids were identified with the unit of % of total fatty acids. PLs were separated

	CA, Washington County, MD, and Allegheny County, Pittsburgh, PA, in 1989. An additional 687 African Americans were recruited in 1992 and later on. The participants recruited were a random sample from the eligibility lists of Medicare from the Health Care Financing Administration (49).	contemporary ATS criteria (51). Due to the timing of omega-3 fatty acid measurements, the spirometry values from year 6 were used for the current analysis.	from other lipids via 1-dimensional TLC. The fatty acids in PLs were trans-methylated and separated by 5890 GC (Agilent Technologies, Palo Alto, CA), with a SP-2560 fused-silica 100-m capillary column and a flame ionization detector. CVs of EPA, DPA, and DHA were all <3%. Measurement of plasma fatty acids across different time points showed that 6-year correlations with baseline EPA, DPA and DHA were 0.55, 0.67, and 0.82, respectively (10).
FHS (Offspring)	Two Framingham Heart Study (FHS) generation cohorts were included in our study, the Offspring cohort and the Third Generation (Gen3) cohort. FHS is a U.S. family-based cohort in Framingham, MA, established in 1948. The Offspring cohort began in 1971 and is comprised of children of the original cohort and spouses of these children (52). Overall, 99.7% participants across all three generations were self-reported Caucasians (53, 54).	Spirometry testing was conducted at each exam, and 1994 ATS criteria was followed (55). In the 7th examination of the Offspring cohort, a 6-L water-filled Collins survey spirometer was used (Warren E. Collins Inc., Braintree, MA), connected to an S&M Instruments software (Doylestown, PA), and the spirometer was calibrated daily (56). Since Exam 8 of the Offspring Cohort, some participants in each cohort underwent post-bronchodilator spirometry testing, besides the regular spirometry testing that all participants performed, so as to differentiate participants with asthma (reversible disease) from those with COPD (fixed disease).	Red blood cells (RBCs) were separated from the whole blood after about a 12-hour fast and was stored at -80℃. Fatty acid composition in RBC was measured using the HS-Omega-3 index® methodology (57). Fatty acid methyl esters were first generated from RBCs and then separated by a CG2010 GC (Shimadzu Corporation, Columbia, MD), with a SP2560 100-m column. The concentration was expressed as % of total identified fatty acids. The inter-assay CV for both EPA and DHA was 4.9%. Omega-3 index (RBC EPA+DHA, expressed as weight % of total fatty acids) was also derived in FHS (58).
FHS (Gen3)	Two Framingham Heart Study (FHS) generation cohorts were included in our study, the	Spirometry testing was conducted at each exam, and 1994 ATS criteria was followed (55). In Exam 2 of the Generation 3	Same as above.

	Offspring cohort and the Third Generation (Gen3) cohort. Starting in 2002, 4,095 adults were enrolled in the Gen3 cohort given at least one of their parents were in the Offspring cohort. In addition, 103 parents of the Gen3 cohort participants were enrolled even though they were not in the Offspring cohort. Overall, 99.7% participants across all three generations were self-reported Caucasians (53, 54).	cohort, a dry rolling-sealed spirometer, connect to the CPL System (Warren E. Collins Inc., Braintree, MA), was used to measure pulmonary function, and the spirometer was calibrated daily (56).	
MESA	The Multi-Ethnic Study of Atherosclerosis (MESA) is a population-based cohort consisting of 6,814 white, black, Hispanic and Asian men and women aged 45-84 yrs who were recruited from 6 sites in U.S., including St Paul, MN, Los Angeles, CA, northern Manhattan, NY, Forsyth County, NC, Chicago, IL, and Baltimore City and County, MD, from July 2000 to August 2002 (59, 60). For this study, only whites and blacks were included.	A dry rolling-sealed spirometer, connected to an automated quality control software (Occupational Marketing, Inc., Houston, TX) was used to carry out pulmonary function testing, in accordance with the 2005 ATS/ERS criteria (46). Each participant was required to have 3 or more acceptable maneuvers. A quality score lower than C, from a 5-point was based on a version of the National Lung Health Education Program, was viewed as low. All results were centrally reviewed. (61)	Fasting blood samples were stored at -70℃ (62), and fatty acids in the blood samples were analyzed at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN, USA). First, plasma PLs were extracted using chloroform/methanol, and then different lipids were separated by TLC (63). The fatty acids in PLs were trans-methylated and measured via a Hewlett Packard 5890 GC, configured for a single capillary Varian CP7420 100-m column with a flame ionization detector (64). The concentration was expressed as % of total fatty acids (63).

RS	The Rotterdam Study (RS) is a population-based cohort which sent out invitation to all residents aged ≥ 55 yrs who lived at a Rotterdam suburb in the Netherlands in 1990-1993 for study participation (65). A total of 7,983 men and women were eventually enrolled in the study (~78% of those eligible) (36).	A SpiroPro® portable spirometer (Erich Jaeger GmbH, Hoechberg, Germany) was used to carry out spirometry from 2002 to 2009. The testing was performed by trained technicians, in accordance with ATS/ERS criteria (66). All measures, as collected from pre-bronchodilator testing, were centrally assessed and validated by researchers. (67)	Fasting blood samples were collected. The plasma was stored at -80℃. Fatty acids in the PL fraction were measured. Lipid extraction was conducted based on a standard method (68). First, plasma lipids were extracted using chloroform/methanol. Second, the PL fraction was separated from other lipid fractions using the solid-phase extraction by NH_2 columns. Third, the PL were methylated and fatty acid methyl esters were measured using high-resolution capillary gas-liquid chromatography (Shimadzu GC17A chromatograph; Shimadzu Benelux, 's-Hertogenbosch, NL), equipped with a 50-m fused silica column and a flame ionization detector. The fatty acids were quantified against the recovered amount of fatty acid methyl ester internal standard (19:0). Fatty acids were expressed as both mg/L plasma and % of total fatty acids. A difference between the values in % of total fatty acids also indicates a difference in absolute values (36).

Abbreviations: AGES = Age, Gene, Environment, Susceptibility Study – Reykjavik; ARIC = Atherosclerosis Risk in Communities; ATS = American Thoracic Society; CARDIA = Coronary Artery Risk Development in Young Adults; CE = Cholesterol ester; CHS = Cardiovascular Health Study; CV = Coefficient of variation; DHA = Docosahexaenoic acid; DPA = Docosapentaenoic acid; EPA = Eicosapentaenoic acid; ERS = European Respiratory Society; FA = Fatty acid; FHS (Offspring) = Framingham Heart Study – the Offspring Cohort; FHS (Gen3) = Framingham Heart Study – the Generation 3 Cohort; GC = Gas chromatograph; MESA = Multi-Ethnic Study of Atherosclerosis; PL = Phospholipid; RBC = Red blood cell; RS = Rotterdam Study, TLC = Thin layer chromatography.

Table E5. Time of measurement for primary study variables in each cohort

Cohort	PFTs Collection Years	n-3 PUFA Biomarkers Collection Years	Smoking Status	Pack-Years	Height	Weight	Age
AGES	2002-2004	Concurrent with PFTs*	All the covariates are concurrent with PFTs.				
ARIC	1987-1989 (Baseline)	Concurrent with PFTs	All the covariates are concurrent with PFTs.				
CARDIA	2005-2006 (Year 20)	Concurrent with PFTs	All the covariates are concurrent with PFTs.				
CHS†	1993-1994 (Year 6)	1992-1993 (Year 5)	All the covariates are concurrent with n-3 PUFAs.‡				
FHS (Offspring)	1998-2001 (Exam 7)§/ 2005-2008 (Exam 8)	2005-2008 (Exam 8)	All the covariates are concurrent with PFTs.				
FHS (Gen 3)	2008-2011 (Exam 2)	Concurrent with PFTs	All the covariates are concurrent with PFTs.				
MESA	2004-2006 (Exam 4)	2000-2002 (Exam 1)	All the covariates are concurrent with PFTs.				
RSI	2002-2004 (Exam 4)	1997-1999 (Exam 3)	All the covariates are concurrent with PFTs.				

Abbreviations: AGES = Age, Gene, Environment, Susceptibility Study – Reykjavik; ARIC = Atherosclerosis Risk in Communities; CARDIA = Coronary Artery Risk Development in Young Adults; CHS = Cardiovascular Health Study; FHS (Offspring) = Framingham Heart Study – the Offspring Cohort; FHS (Gen3) = Framingham Heart Study – the Generation 3 Cohort; MESA = Multi-Ethnic Study of Atherosclerosis; n-3 PUFA = Omega-3 poly-unsaturated fatty acid; PFT = Pulmonary function test; RS = Rotterdam Study.

*In AGES, the blood samples were drawn at the time concurrent with PFTs (2002-2004). However, the n-3 PUFA biomarkers were measured from blood samples in 2013.

†In CHS, covariates from year 5 (concurrent with n-3 PUFA biomarkers) were used, but results were similar using covariates from either year 5 or year 6 (concurrent with PFT).

‡In CHS, the initial cohort (European and African ancestry participants) was enrolled in 1989-1990 and an additional cohort (African ancestry participants only) was recruited in 1992 and 1993. For the original cohort, pack-years in year 5 was extrapolated from that at baseline; for the additional cohort, pack-years in year 5 was calculated based on years and amount smoked. In addition, in CHS, height was only measured in year 5, but not in year 6.

§For the Offspring cohort, 2,165 participants had their PFT and other covariates measured at exam 8. However, 4 participants with PFT and other covariates measured at exam 7 were included in this study due to their missing data at exam 8.

Table E6. Participant characteristics averaged across cohorts[*]

Characteristics	Mean (SD) or Percent (%)[‖]	Range of Mean or Percent[**]
No. of Participants	16,134	141 – 3,254
Males, %	45.4	30.9 – 49.6
African ancestry, %	15.5	12.6 – 45.4[††]
Current Smoker, %	14.1	8.0 – 24.1
Former Smoker, %	37.6	13.5 – 57.5
Pack-Years[†]	21 (21)	11 – 30
Age, year	57 (13)	45 – 76
Height, m	1.69 (0.10)	1.64 – 1.72
Weight, kg[‡]	80.0 (18.5)	71.3 – 90.5
FEV_1, mL	2,831 (876)	1,765 – 3,388
FVC, mL	3,786 (1,104)	2,440 – 4,449
FEV_1/FVC (%)	74.7 (8.2)	69.6 – 79.7
ALA (% of total FAs)	0.17 (0.08)	0.14 – 0.23
EPA (% of total FAs)	0.76 (0.65)	0.56 – 2.87
DPA (% of total FAs)		
- Plasma Phospholipids	0.92 (0.20)	0.83 – 1.18
- RBC[‡‡]	2.64 (0.47)	2.55 – 2.76
DHA (% of total FAs)	3.67 (1.41)	2.81 – 6.33
Total n-3 PUFAs (% of total FAs)	6.08 (2.37)	4.41 – 10.60
Time Difference between PFT and n-3 PUFAs, days[§]	266 (580)	0 – 1,769

Abbreviations: AA = African ancestry; AGES = Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ALA = Alpha-linolenic acid; ARIC = Atherosclerosis Risk in Communities; CARDIA = Coronary Artery Risk Development in Young Adults; CHS = Cardiovascular Health Study; DHA = Docosahexaenoic acid; DPA = Docosapentaenoic acid; EA = European ancestry; EPA = Eicosapentaenoic acid; FA = Fatty acid; FEV_1 = Forced expiratory volume in the first second; FHS (Offspring) = Framingham Heart Study—Offspring Cohort; FHS (Gen3) = Framingham Heart Study—Generation 3 Cohort; FVC = Forced vital capacity; MESA = Multi-Ethnic Study of Atherosclerosis; n-3 PUFA = Omega-3 poly-unsaturated fatty acid; PFT = Pulmonary function test; RBC = Red blood cell; RS = Rotterdam (Netherlands) Study; SD = Standard Deviation.

[*]Data are presented as mean (SD) unless otherwise indicated. This table corresponds to the n-3 PUFA-PFT association analyses and thus includes all seven cohorts. The total sample size was 16,134 for the meta-analysis of omega-3 fatty acid biomarkers, in which 13,629 are European ancestry participants, and 2,505 are African ancestry participants. All the cohorts measured n-3 PUFAs in plasma phospholipids, except FHS, which measured n-3 PUFAs in red blood cells.

[†]Descriptive statistics of pack-years among ever smokers.

[‡]Number of participants with weight data was slightly different from the number of participants shown in this table for some cohorts. In CHS, 242 out of 243 AAs and 1,687 out of 1,690 EAs had weight data. In CARDIA, all 1,759 EAs and 1,456 out of 1,461 AAs had weight data.

[§]The time difference refers to the interval between measurement of PFT and n-3 PUFAs. If the difference is positive, n-3 PUFAs were measured before PFTs; if it is negative, n-3 PUFAs were measured after PFTs.

[ll]The mean (SD) or percentage was weighted by the sample size of each cohort.

[**]Range is presented for each ancestry-specific cohort, therefore 11 cohorts/sub-cohorts are included here (AGES-EA, ARIC-EA, CARDIA-EA, CARDIA-AA, CHS-EA, CHS-AA, MESA-EA, MESA-AA, RS-EA, FHS-Offspring-EA, and FHS-Gen3-EA).

[††]Only CARDIA, CHS, and MESA contributed to the statistical analysis for AA participants and thus contributed to the percentages of AA participants here.

[‡‡]Only FHS-Offspring and FHS-Gen3 have DPA measured in RBCs.

Table E7. Omega-3 fatty acid biomarker levels by smoking status in each cohort

Cohorts	Mean ALA biomarker level (% of total fatty acids)			Mean EPA biomarker level (% of total fatty acids)			Mean DPA biomarker level (% of total fatty acids)			Mean DHA biomarker level (% of total fatty acids)		
	Never smoker	Former smoker	Current smoker	Never smoker	Former smoker	Current smoker	Never smoker	Former smoker	Current smoker	Never smoker	Former smoker	Current smoker
AGES-EA (N=424)	0.22	0.23	0.24	2.98	2.84	2.53	1.18	1.18	1.13	6.45	6.34	5.85
ARIC-EA (N=3,143)	0.15	0.14	0.14	0.57	0.58	0.53	0.92	0.90	0.88	2.94	2.85	2.54
CARDIA-EA (N=1,759)	0.19	0.20	0.19	0.87	0.86	0.73	0.95	0.93	0.93	3.18	3.19	2.59
CARIDA-AA (N=1,461)	0.17	0.17	0.17	0.70	0.71	0.63	0.93	0.91	0.93	3.39	3.48	2.94
CHS-EA (N = 1690)	0.15	0.15	0.14	0.58	0.62	0.58	0.84	0.84	0.79	2.94	3.05	2.76
CHS-AA (N = 243)	0.13	0.14	0.13	0.59	0.65	0.56	0.86	0.86	0.87	3.57	3.53	3.58
MESA-EA (N = 1140)	0.18	0.18	0.18	0.93	0.97	0.71	0.95	0.93	0.88	3.63	3.56	2.86
MESA-AA (N = 801)	0.16	0.15	0.16	0.96	0.90	0.76	0.97	0.94	0.91	4.46	4.30	3.67
RS-EA (N = 141)	0.18	0.16	0.15	0.81	0.87	0.85	0.94	0.94	0.94	3.63	3.52	3.32
FHS-Offspring-EA* (N = 2,169)	0.18	0.18	0.18	0.75	0.76	0.66	2.78	2.77	2.62	4.99	4.90	4.09
FHS-Gen3-EA* (N = 3,052)	0.17	0.17	0.16	0.66	0.71	0.57	2.56	2.57	2.49	4.29	4.24	3.43

Abbreviations: AA = African ancestry; AGES = Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ALA = Alpha-linolenic acid; ARIC = Atherosclerosis Risk in Communities; CARDIA = Coronary Artery Risk Development in Young Adults; CHS = Cardiovascular Health Study; DHA = Docosahexaenoic acid; DPA = Docosapentaenoic acid; EA = European ancestry; EPA = Eicosapentaenoic acid; FHS (Offspring) = Framingham Heart Study—Offspring Cohort; FHS (Gen3) = Framingham Heart Study—Generation 3 Cohort; MESA = Multi-Ethnic Study of Atherosclerosis; RS = Rotterdam (Netherlands) Study.

*Omega-3 fatty acid biomarkers were measured in plasma in all cohorts, except those in FHS (measured in red blood cells).

Table E8. Meta-analysis results for the primary analyses of each omega-3 fatty acid biomarker on each pulmonary function test measure*

	FEV_1				**FVC**				**FEV_1/FVC (in percent)**			
n-3 PUFA (Ancestry)	**β (mL)**	**SE**	**P-value**	***Effect per 1 SD increment*** §	**β (mL)**	**SE**	**P-value**	***Effect per 1 SD increment*** §	**β (%)**	**SE**	**P-value**	***Effect per 1 SD increment*** §
DHA (All)	14.3†	3.2	**<0.001**	20.3	8.4	3.7	**0.023**	11.9	0.03†	0.05	0.478	0.05
DHA (EA)	*15.4*	*3.4*	***<0.001***	*21.8*	*9.7*	*4.0*	***0.016***	*13.7*	*0.01†*	*0.05*	*0.816*	*0.02*
DHA (AA)	*8.4‡*	*8.1*	*0.300*	*11.8*	*1.3†*	*9.5*	*0.890*	*1.9*	*0.16†*	*0.12*	*0.192*	*0.23*
DPA (All)	39.3†	11.5	**<0.001**	8.1	32.4‡	13.3	**0.015**	6.7	-0.2	0.2	0.238	-0.04
DPA (EA)	*39.6‡*	*11.9*	***<0.001***	*8.1*	*35.7‡*	*13.8*	***0.010***	*7.3*	*-0.2*	*0.2*	*0.244*	*-0.04*
DPA (AA)	*35.6*	*41.0*	*0.384*	*7.3*	*-6.3*	*48.0*	*0.895*	*-1.3*	*-0.1*	*0.6*	*0.825*	*-0.03*
EPA (All)	2.9	6.6	0.658	1.9	2.8	7.7	0.711	1.9	-0.2	0.1	**0.016**	-0.16
EPA (EA)	*3.4*	*7.2*	*0.638*	*2.2*	*4.7*	*8.3*	*0.573*	*3.1*	*-0.3*	*0.1*	***0.007***	*-0.19*
EPA (AA)	*0.41*	*16.8*	*0.981*	*0.3*	*-7.5*	*19.7*	*0.703*	*-4.9*	*0.04*	*0.27*	*0.868*	*0.03*
ALA (All)	81.9	45.6	0.072	6.5	120.5	53.1	**0.023**	9.5	-2.9†	0.7	**<0.001**	-0.23
ALA (EA)	*54.3*	*49.1*	*0.270*	*4.3*	*95.4*	*57.3*	*0.096*	*7.5*	*-2.6‡*	*0.7*	***<0.001***	*-0.20*
ALA (AA)	*250.7*	*121.5*	***0.039***	*19.8*	*272.2†*	*140.8*	*0.053*	*21.5*	*-4.3*	*1.7*	***0.010***	*-0.34*

Abbreviations: AA = African ancestry; ALA = Alpha-linolenic acid; DHA = Docosahexaenoic acid; DPA = Docosapentaenoic acid; EPA = Eicosapentaenoic acid; FEV_1 = Forced expiratory volume in the first second; FVC = Forced vital capacity; n-3 PUFA = Omega-3 poly-unsaturated fatty acid; SD = Standard deviation; SE = Standard error.

[*]The β coefficient corresponds to the association of each n-3 PUFA biomarker on the specific pulmonary function outcome from a meta-analysis across cohorts [individual cohort analyses adjusted for current and former smoking, with never smoking as the reference, pack-years, sex, age, age^2, height, height^2, weight (for the FVC outcome only), and study site (cohorts with >1 site)]. P-values that are ≤0.05 are bolded.

[†]Moderate heterogeneity with I^2 ranging from 33% to 60%.

[‡]Substantial heterogeneity with I^2 ranging from 62% to 82%.

[§]One standard deviation increments of DHA (all cohorts), DPA (all except FHS), EPA (all cohorts), and ALA (all cohorts) biomarkers are 1.41%, 0.20%, 0.65%, and 0.08% of total fatty acids, respectively.

Table E9. Meta-analysis results of the interaction of each omega-3 fatty acid biomarker and smoking status on each pulmonary function test measure[*]

n-3 PUFA	Smoking status (vs. never smoking as reference)	FEV_1				FVC				FEV_1/FVC (in percent)			
		β (mL)	SE	P-value	*Effect per 1 SD increment*[§]	β (mL)	SE	P-value	*Effect per 1 SD increment*[§]	β (%)	SE	P-value	*Effect per 1 SD increment*[§]
DHA	*Current*	26.1	11.1	**0.019**	36.8	9.5	12.9	0.463	13.4	0.9	0.2	**<0.001**	1.2
	Former	3.5[†]	6.7	0.596	5.0	-0.6	7.7	0.937	-0.9	0.1	0.1	0.158	0.2
DPA	*Current*	44.8	40.4	0.268	9.0	43.3	46.6	0.353	8.7	1.1	0.6	0.060	0.2
	Former	39.3	24.1	0.104	7.9	30.0	27.9	0.282	6.0	0.4	0.4	0.208	0.1
EPA	*Current*	34.4	25.1	0.171	22.4	19.1	29.1	0.513	12.4	0.6	0.4	0.110	0.4
	Former	2.8[†]	14.1	0.841	1.8	-1.8[†]	16.4	0.912	-1.2	-0.0004	0.2222	0.999	-0.0003
ALA	*Current*	-109.1	144.1	0.449	-8.7	-151.0	167.6	0.367	-12.1	-0.09	2.06	0.965	-0.01
	Former	-147.2	106.4	0.167	-11.8	-68.2	123.5	0.581	-5.5	-2.2	1.6	0.169	-0.2

Abbreviations: ALA = Alpha-linolenic acid; DHA = Docosahexaenoic acid; DPA = Docosapentaenoic acid; EPA = Eicosapentaenoic acid; FEV_1 = Forced expiratory volume in the first second; FVC = Forced vital capacity; n-3 PUFA = Omega-3 poly-unsaturated fatty acid; SD = Standard deviation; SE = Standard error.

[*]Fixed effect models were used. β (mL) and β (%) were the coefficients of the interaction terms only (the interaction of current smoking status with the n-3 PUFA biomarkers, and the interaction of former smoking status with the n-3 PUFA biomarkers), with never smokers as the reference group. P-values that are ≤0.05 are bolded. Coefficients were meta-analyzed across cohorts, and each cohort's analysis was adjusted for the following covariates: current and former smoking, with never smoking as the reference, pack-years, sex, age, age^2, height, height^2, weight (for the FVC outcome only), and study site (cohorts with >1 site).

[†]Moderate heterogeneity with I^2 ranging from 30% to 60%.

[§]One standard deviation increments of DHA (all cohorts), DPA (all except FHS), EPA (all cohorts), and ALA (all cohorts) biomarkers are 1.41%, 0.20%, 0.65%, and 0.08% of total fatty acids, respectively.

Table E10. Previously reported lead SNPs at genome-wide significant loci for either of the omega-3 fatty acid biomarkers (DHA or DPA) and their associations with FEV_1 and FVC in the current genome-wide joint 2df meta-analyses that included interaction with the n-3 fatty acid biomarkers (DHA or DPA)

Chr	Position	SNP	Gene / nearby genes	Prior GWAS phenotype	Prior GWAS P	DHA interaction model for FEV_1			DPA interaction model for FEV_1			DHA interaction model for FVC			DPA interaction model for FVC		
						P_{main}	P_{int}	P_{2df}	P_{main}	P_{int}	P_{2df}	P_{main}	P_{int}	P_{2df}	P_{main}	P_{int}	P_{2df}
6	10994782	rs2236212	*ELOVL2*	DHA	1.0×10^{-15}	0.95	0.92	0.99	0.51	0.89	0.54	0.30	0.21	0.42	0.11	0.22	0.26
6	10968908	rs4713103	*SYCP2L*	DHA/ DPA	8.0×10^{-14} / 3.0×10^{-36}	0.80	0.92	0.90	0.76	0.51	0.70	0.67	0.44	0.54	0.54	0.62	0.83
6	11074114	rs4711171	*ELOVL2-AS1*	DHA	5.0×10^{-13}	0.68	0.62	0.87	0.36	0.58	0.59	0.11	0.062	0.16	0.093	0.15	0.24
2	27518370	rs780094	*GCKR*	DPA	9.0×10^{-9}	0.48	0.47	0.76	0.74	0.74	0.94	0.45	0.50	0.75	0.67	0.75	0.91
6	10982740	rs3734398	*ELOVL2*	DPA	1.0×10^{-43}	0.68	0.67	0.91	0.59	0.98	0.58	0.56	0.47	0.74	0.12	0.25	0.29
6	11075793	rs1321535	*ELOVL2-AS1*	DPA	1.0×10^{-38}	0.62	0.54	0.81	0.43	0.65	0.65	0.15	0.080	0.20	0.10	0.17	0.27
11	61723014	rs198426	*DAGLA*	DPA	3.0×10^{-9}	0.39	0.73	0.31	0.040	0.18	0.080	0.52	0.99	0.21	0.063	0.33	0.079
11	61783884	rs174535	*MYRF*	DPA	1.0×10^{-151}	0.84	0.56	0.57	0.24	0.96	0.095	0.59	0.35	0.44	0.12	0.67	0.063
11	61790331	rs102275	*TMEM258*	DPA	8.0×10^{-153}	0.94	0.57	0.23	0.030	0.49	8.7×10^{-3}	0.81	0.44	0.36	0.040	0.45	0.018
11	61796827	rs4246215	*FEN1*	DPA	1.0×10^{-139}	0.95	0.57	0.26	0.064	0.69	0.016	0.96	0.46	0.21	0.026	0.43	7.6×10^{-3}
11	61803311	rs174547	*FADS1*	DPA	4.0×10^{-154}	0.70	0.37	0.34	0.086	0.65	0.037	0.55	0.27	0.31	0.066	0.53	0.033
11	61830500	rs1535	*FADS2*	DPA	3.0×10^{-152}	0.98	0.61	0.45	0.055	0.49	0.034	0.83	0.48	0.41	0.036	0.38	0.024
11	61872101	rs174448	*FADS2/ FADS3*	DPA	3.0×10^{-60}	0.83	0.43	0.29	0.052	0.37	0.059	0.48	0.25	0.35	0.12	0.49	0.14
11	61896219	rs174468	*FADS3/ RAB3IL1*	DPA	3.0×10^{-35}	0.10	0.37	0.054	2.2×10^{-3}	0.091	**1.8×10^{-3}**	0.16	0.52	0.083	0.010	0.27	3.9×10^{-3}
11	61944003	rs2521572	*RAB3IL1*	DPA	2.0×10^{-9}	0.052	0.033	0.10	0.79	0.67	0.89	0.033	0.053	0.10	0.90	0.75	0.71

Abbreviations: chr = Chromosome; DHA = Docosahexaenoic acid; DPA = Docosapentaenoic acid; FEV_1 = Forced expiratory volume in the first second; FVC = Forced vital capacity; GWAS = Genome-wide association study; int = Interaction; NA = Not available; SNP = Single nucleotide polymorphism.

*SNPs with the smallest P values from each locus reported in the prior GWAS analysis (69) are shown and are sorted by DHA or DPA phenotype and then by chromosomal position (NCBI build 37). The SNP that passed the multiple testing correction (*n* of tests = 14, Bonferroni-corrected $P<3.6\times10^{-3}$) is bolded.

Table E11. Previously reported lead SNPs at genome-wide significant loci for any of the pulmonary function test measures (FEV_1, FVC, or FEV_1/FVC) and their associations in the current genome-wide joint 2df meta-analyses that included interaction with the n-3 fatty acid biomarkers (DHA or DPA)[*]

chr	Position	SNP	Gene / nearby genes	Prior GWAS phenotype	Prior GWAS best P	DHA interaction model for FEV_1			DPA interaction model for FEV_1			DHA interaction model for FVC			DPA interaction model for FVC		
						P_{main}	P_{int}	P_{2df}	P_{main}	P_{int}	P_{2df}	P_{main}	P_{int}	P_{2df}	P_{main}	P_{int}	P_{2df}
1	150586971	rs6681426	*MCL1/ENSA*	FEV_1	4.4×10^{-9} (70)	0.25	0.80	0.054	0.13	0.71	0.062	0.020	0.15	0.013	0.049	0.52	0.019
1	204434927	rs12092943	*PIK3C2B*	FEV_1	4.8×10^{-8} (71)	0.057	0.22	0.055	0.060	0.34	0.071	0.15	0.51	0.064	0.073	0.44	0.060
1	221765779	1:221765779: C_CA	*C1orf140/ DUSP10*	FEV_1	3.4×10^{-8} (71)	0.56	0.69	0.69	0.76	0.58	0.54	0.69	0.68	0.92	0.45	0.41	0.71
1	237941781	rs3766889	*RYR2*	FEV_1	4.1×10^{-8} (71)	6.3×10^{-4}	5.6×10^{-3}	6.4×10^{-4}	0.24	0.56	0.022	0.021	0.099	9.9×10^{-3}	0.13	0.33	0.025
2	42355947	rs963406	*PKDCC/ EML4*	FEV_1	3.2×10^{-8} (71)	0.56	0.95	0.20	0.28	0.86	0.15	0.27	0.55	0.24	0.11	0.37	0.16
2	218683154	rs2571445	*TNS1*	FEV_1	1.1×10^{-12} (72)	0.081	0.99	**6.0×10^{-6}**	2.4×10^{-3}	0.52	**5.5×10^{-6}**	0.57	0.20	**1.3×10^{-5}**	0.021	1.00	**3.5×10^{-5}**
3	57494433	rs79294353	*DNAH12*	FEV_1	4.8×10^{-9} (71)	0.41	0.65	0.50	0.84	0.66	0.46	0.59	0.61	0.86	0.70	0.72	0.93
3	98815640	rs6778584	*DCBLD2/ MIR548G*	FEV_1	4.5×10^{-8} (71)	0.27	0.35	0.52	0.046	0.037	0.11	0.074	0.16	0.15	0.010	0.020	0.036
3	169300219	rs1344555	*MECOM*	FEV_1	2.7×10^{-8} (73)	0.57	0.76	0.69	0.52	0.82	0.65	0.31	0.22	0.43	0.31	0.16	0.35
4	106137033	rs2047409	*TET2*	FEV_1	1.3×10^{-8} (74)	0.21	0.51	0.14	0.17	0.66	0.15	0.49	0.80	0.44	0.21	0.49	0.34
4	106531846	rs17035960	*FLJ20184*	FEV_1	9.4×10^{-14} (9)	0.11	0.83	**3.3×10^{-6}**	0.14	0.28	**6.9×10^{-6}**	0.10	0.81	4.2×10^{-4}	0.17	0.48	2.8×10^{-4}
4	106563379	rs17036052	*FLJ20184*	FEV_1	1.8×10^{-15} (9)	0.13	0.85	**1.1×10^{-5}**	0.19	0.29	**1.4×10^{-5}**	0.17	0.93	1.9×10^{-3}	0.29	0.42	9.0×10^{-4}
4	106593574	rs17036090	*FLJ20184*	FEV_1	5.6×10^{-15} (9)	0.23	0.57	**9.8×10^{-6}**	0.34	0.12	**9.7×10^{-6}**	0.17	0.89	2.2×10^{-3}	0.22	0.50	1.2×10^{-3}
4	106619140	rs11727189	*INTS12*	FEV_1	4.7×10^{-17} (9)	0.17	0.74	**2.1×10^{-5}**	0.19	0.33	**3.5×10^{-5}**	0.18	0.95	2.3×10^{-3}	0.14	0.71	1.8×10^{-3}

4	106688904	rs10516526	*GSTCD*	FEV_1	2.2×10^{-23} (72)	0.13	0.80	**9.6×10^{-6}**	0.14	0.38	**2.1×10^{-5}**	0.15	0.91	1.7×10^{-3}	0.12	0.76	1.3×10^{-3}
4	106729933	rs11097901	*GSTCD*	FEV_1	3.3×10^{-18} (9)	0.083	0.97	**1.4×10^{-5}**	0.18	0.30	**2.0×10^{-5}**	0.075	0.59	2.1×10^{-3}	0.15	0.66	1.5×10^{-3}
4	106755996	rs11728716	*GSTCD*	FEV_1	7.2×10^{-18} (9)	0.18	0.69	**1.1×10^{-5}**	0.11	0.49	**3.1×10^{-5}**	0.18	0.90	4.0×10^{-3}	0.17	0.73	3.5×10^{-3}
4	106796829	rs17036341	*GSTCD/ NPNT*	FEV_1	2.2×10^{-15} (9)	0.023	0.38	**1.5×10^{-4}**	0.57	0.11	**1.8×10^{-4}**	0.056	0.33	0.012	0.45	0.43	0.015
4	106808107	rs17331332	*GSTCD/ NPNT*	FEV_1	5.7×10^{-15} (9, 72)	0.10	0.93	**1.3×10^{-5}**	0.11	0.51	**3.1×10^{-5}**	0.17	0.85	4.6×10^{-3}	0.19	0.68	3.9×10^{-3}
4	106819053	rs34712979	*NPNT*	FEV_1	9.6×10^{-16} (74)	0.061	0.34	0.022	0.012	0.16	0.014	0.28	0.57	0.32	0.037	0.13	0.093
4	146174040	rs111898810	*OTUD4/ SMAD1*	FEV_1	2.1×10^{-8} (71)	0.22	0.054	0.050	0.79	0.32	0.33	0.18	0.051	0.072	0.95	0.54	0.56
5	55922145	rs11748173	*ANKRD55/ MAP3K1*	FEV_1	3.9×10^{-10} (71)	0.50	0.73	0.57	0.43	0.76	0.56	0.61	0.86	0.66	0.88	0.43	0.45
5	77392117	rs252746	*AP3B1*	FEV_1	6.2×10^{-9} (71)	0.80	0.66	0.83	0.12	0.081	0.22	0.80	0.74	0.94	0.43	0.49	0.73
5	147845815	rs3995090	*HTR4*	FEV_1	4.3×10^{-9} (72)	8.5×10^{-4}	6.4×10^{-3}	2.2×10^{-3}	0.068	0.61	0.023	0.025	7.8×10^{-3}	0.024	0.75	0.58	0.82
5	147846707	rs6889822	*HTR4*	FEV_1	8.2×10^{-9} (72)	6.2×10^{-4}	6.5×10^{-3}	1.3×10^{-3}	0.015	0.29	7.6×10^{-3}	0.042	0.025	0.081	0.40	0.39	0.68
5	148596693	rs3839234	*ABLIM3*	FEV_1	4.5×10^{-11} (75)	0.54	0.35	0.53	0.50	0.64	0.77	1.00	0.79	0.78	0.62	0.83	0.81
6	28322296	rs6903823	*ZKSCAN3/ ZNF323*	FEV_1	2.2×10^{-10} (73)	0.91	0.42	0.15	0.84	0.28	0.18	0.94	0.71	0.72	0.61	0.38	0.58
6	32635592	rs9274600	*HLA-DQB1/ HLA-DQA3*	FEV_1	1.3×10^{-10} (74)	0.40	0.28	0.50	0.44	0.42	0.71	0.42	0.42	0.72	0.24	0.24	0.50
6	32648418	rs114229351[†]	*HLA-DQB1/ HLA-DQA2*	FEV_1	2.1×10^{-10} (75)	0.065	0.10	0.18	0.082	0.16	0.22	0.088	0.070	0.19	0.14	0.090	0.24
7	156127246	rs12698403	*LOC389602/ LOC285889*	FEV_1	1.1×10^{-13} (75)	0.84	0.39	0.16	0.85	0.54	0.30	0.67	0.21	0.050	0.75	0.40	0.093
9	4124377	rs7872188	*GLIS3*	FEV_1	1.6×10^{-10} (75)	0.24	0.62	0.16	0.057	0.29	0.091	0.13	0.43	0.080	0.013	0.12	0.026

10	65087468	rs7899503	*JMJD1C*	FEV_1	8.7×10^{-14} (71)	0.037	0.24	8.9×10^{-3}	0.028	0.47	5.4×10^{-3}	0.30	0.89	0.035	0.080	0.66	0.026
10	78315224	rs11001819	*C10orf11*	FEV_1	3.0×10^{-12} (73)	0.46	0.13	0.053	0.97	0.22	0.070	0.99	0.48	0.14	0.93	0.29	0.083
11	62310909	rs2509961	*AHNAK*	FEV_1	1.5×10^{-13} (75)	0.16	0.21	0.37	0.30	0.41	0.58	0.14	0.17	0.34	0.49	0.66	0.75
11	86442733	rs145729347	*ME3/PRSS23*	FEV_1	8.6×10^{-9} (75)	0.24	0.66	0.13	0.67	0.63	0.21	0.85	0.70	0.85	0.99	0.95	0.99
11	126008910	rs567508	*CDON/ RPUSD4*	FEV_1	4.8×10^{-10} (75)	0.63	0.28	0.20	0.85	0.28	0.21	0.63	0.96	0.47	0.93	0.36	0.25
12	56390364	rs772920	*RAB5B*	FEV_1	2.5×10^{-8} (71)	0.87	0.80	0.95	0.44	0.30	0.57	0.48	0.67	0.63	0.92	0.65	0.57
12	65824670	rs1494502	*MSRB3*	FEV_1	9.8×10^{-10} (75)	0.044	0.069	0.13	0.48	0.80	0.59	0.31	0.39	0.58	0.68	0.93	0.80
12	114743533	chr12: 114743533	*RBM19/ TBX5*	FEV_1	1.2×10^{-8} (74)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
12	115201436	rs10850377	*TBX3*	FEV_1	2.5×10^{-12} (70)	0.60	0.96	0.28	0.88	0.24	0.12	0.58	0.86	0.12	0.36	0.82	0.084
12	125230287	rs11057793	*NCOR2\ SCARB1*	FEV_1	4.8×10^{-8} (71)	0.57	0.87	0.53	0.20	0.47	0.34	0.77	0.86	0.46	0.26	0.61	0.36
14	92485881	rs7155279	*TRIP11*	FEV_1	1.4×10^{-9} (70)	0.75	0.46	0.46	0.17	0.28	0.38	0.46	0.25	0.35	0.086	0.14	0.23
14	93118229	rs117068593	*RIN3*	FEV_1	2.3×10^{-8} (70)	0.21	0.76	0.029	0.31	0.65	0.020	0.44	0.80	0.33	0.75	0.63	0.33
17	29087285	rs62070631	*SUZ12P1*	FEV_1	2.6×10^{-8} (71)	0.070	0.38	0.021	0.11	0.80	0.029	0.30	0.74	0.16	0.25	0.78	0.20
17	43682323	rs186806998	*LOC644172/ CRHR1*	FEV1	3.5×10^{-10} (71)	0.19	0.95	4.2×10^{-3}	0.10	0.93	3.4×10^{-3}	0.21	0.88	9.7×10^{-3}	0.020	0.40	5.2×10^{-3}
17	43685698	rs143246821		FEV_1	9.1×10^{-10} (71)	0.082	0.61	3.1×10^{-3}	0.046	0.72	2.2×10^{-3}	0.11	0.59	9.8×10^{-3}	8.6×10^{-3}	0.20	3.7×10^{-3}
17	44339473	rs2532349	*KANSL1*	FEV_1	1.7×10^{-10} (70)	0.13	0.81	3.2×10^{-3}	0.056	0.80	2.4×10^{-3}	0.15	0.77	7.6×10^{-3}	0.012	0.28	3.8×10^{-3}
17	44847834	rs199525	*WNT3*	FVC, FEV_1	9.6×10^{-10} (71)	0.50	0.58	6.0×10^{-3}	0.098	0.93	6.1×10^{-3}	0.49	0.78	0.039	0.026	0.28	0.020

17	44863133	rs916888		FEV_1	3.8×10^{-9} (71)	0.81	0.39	0.014	0.32	0.67	0.024	0.96	0.31	0.033	0.096	0.54	0.070
17	69125606	rs11654749	*KCNJ2/ SOX9*	FEV_1	1.3×10^{-8} (4)	0.53	0.65	0.016	0.21	0.88	0.031	0.83	0.32	0.10	0.67	0.29	0.12
17	73513185	rs7218675	*TSEN54*	FEV_1	1.2×10^{-8} (74)	0.20	0.34	0.34	0.11	0.25	0.26	0.064	0.23	0.061	3.0×10^{-3}	0.022	0.010
18	8801351	rs513953	*SOGA2*	FEV_1	2.0×10^{-8} (71)	0.012	0.084	0.011	0.30	0.80	0.039	0.063	0.12	0.16	0.16	0.35	0.34
18	20148531	rs7243351	*CTAGE1/ RBBP8*	FEV_1	4.7×10^{-8} (71)	0.72	0.48	0.017	0.011	0.15	0.011	0.66	0.26	0.13	0.077	0.27	0.14
20	25669052	rs6138639	*ZNF337*	FEV_1	3.2×10^{-10} (71)	9.5×10^{-3}	0.20	3.0×10^{-4}	0.10	0.79	1.1×10^{-3}	0.014	0.15	2.0×10^{-3}	0.039	0.58	5.7×10^{-3}
20	31042176	rs1737889	*C20orf112*	FEV_1	4.2×10^{-8} (71)	0.16	0.31	0.25	0.64	0.80	0.39	0.39	0.72	0.33	0.15	0.43	0.24
20	62363640	rs72448466	*ZGPAT*	FEV_1	4.3×10^{-13} (75)	0.66	0.86	0.22	0.62	0.69	0.19	0.55	0.91	0.40	0.89	0.58	0.39
22	18450287	rs11704827	*MICAL3*	FEV_1	8.3×10^{-13} (75)	0.29	0.48	0.44	0.74	0.82	0.58	0.71	0.90	0.79	0.48	0.66	0.75
22	28056338	rs134041	*MN1*	FEV_1	3.0×10^{-9} (70)	0.28	0.39	0.52	0.86	0.77	0.65	0.65	0.86	0.72	0.76	0.88	0.68
22	28181399	rs2283847	*MN1*	FEV_1	3.4×10^{-11} (75)	0.87	0.80	0.48	0.38	0.27	0.34	0.65	0.89	0.62	0.59	0.44	0.45
11	86376739	rs507211	*ME3*	longitu-dinal FEV_1	2.2×10^{-8} (76)	1.00	0.58	0.33	0.20	0.58	0.23	0.54	0.98	0.31	0.56	0.77	0.24
1	118862070	rs200154334	*SPAG17/ TBX15*	FVC	8.2×10^{-14} (75)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1	146494027	rs12724426	*LOC728989*	FVC	3.0×10^{-8} (71)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1	200085714	rs2821332	*NR5A2*	FVC	7.7×10^{-9} (71)	0.91	0.86	0.73	0.97	0.65	0.68	0.99	0.83	0.83	0.57	0.76	0.80
1	215095003	rs512597	*CENPF/ KCNK2*	FVC	3.9×10^{-9} (71)	0.61	0.80	0.72	0.22	0.31	0.47	0.35	0.77	0.18	0.15	0.49	0.20
1	221630555	rs6657854	*C1orf140/ DUSP10*	FVC	1.2×10^{-8} (71)	0.26	0.77	0.069	0.10	0.56	0.067	0.13	0.47	0.053	0.15	0.75	0.056

1	221635207	rs12046746		FVC	1.4×10^{-9} (71)	0.26	0.78	0.069	0.10	0.56	0.068	0.13	0.47	0.052	0.15	0.76	0.055
1	237929787	1:237929787:T_TCA	*RYR2*	FVC	4.5×10^{-8} (71)	0.59	0.36	0.43	0.14	0.23	0.33	0.59	0.37	0.46	0.16	0.24	0.38
2	56120853	rs1430193	*EFEMP1*	FVC	1.9×10^{-12} (77)	0.12	0.35	0.14	0.096	0.37	0.14	0.074	0.46	0.010	0.053	0.55	0.014
2	109571508	rs17034666	*EDAR*	FVC	1.8×10^{-8} (71)	0.34	0.24	0.48	0.54	0.52	0.81	0.082	0.065	0.18	0.94	0.99	0.99
2	119660943	rs114962105	*EN1/ MARCO*	FVC	3.8×10^{-8} (71)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	147046592	rs6746679	*DKFZp686O1327/ PABPC1P2*	FVC	2.2×10^{-8} (71)	0.18	0.15	0.36	0.96	0.89	0.98	0.13	0.16	0.32	0.59	0.77	0.82
3	67452043	rs1490265	*SUCLG2*	FVC	1.6×10^{-9} (75)	0.15	0.17	0.36	0.68	0.85	0.86	0.70	0.78	0.90	0.67	0.50	0.76
3	98806782	rs1404098	*DCBLD2/ MIR548G*	FVC	5.5×10^{-9} (71)	0.38	0.50	0.63	0.044	0.037	0.10	0.10	0.24	0.17	7.5×10^{-3}	0.017	0.028
3	158282459	rs6441207	*RSRC1/ MLF1*	FVC	1.3×10^{-13} (70)	0.27	0.79	0.066	0.36	0.86	0.11	0.089	0.18	0.20	0.75	0.73	0.49
5	33334312	rs91731	*LOC340113/ TARS*	FVC	4.3×10^{-13} (75)	0.84	0.75	0.36	0.70	0.79	0.47	0.63	0.95	0.30	0.26	0.58	0.39
5	53444498	rs2441026	*ARL15*	FVC	2.8×10^{-12} (75)	0.27	0.43	0.44	0.49	0.84	0.60	0.34	0.46	0.57	0.88	0.57	0.67
5	77450828	rs12513481	*AP3B1*	FVC	2.2×10^{-11} (71)	0.44	0.38	0.67	0.16	0.14	0.32	0.96	0.93	0.93	0.43	0.54	0.72
5	77440196	rs72776440		FVC	3.2×10^{-11} (71)	0.62	0.50	0.75	0.18	0.13	0.32	0.97	1.00	1.00	0.55	0.58	0.83
6	7801112	rs6923462	*BMP6*	FVC	5.9×10^{-13} (77)	0.13	0.29	0.21	0.58	0.76	0.28	0.18	0.57	0.074	0.89	0.13	0.025
6	126792095	rs11759026	*CENPW/ RSPO3*	FVC	4.3×10^{-9} (71)	0.69	0.31	0.22	0.68	0.19	0.15	0.45	0.99	0.13	0.81	0.33	0.067
7	15506529	rs55905169	*AGMO*	FVC	1.3×10^{-8} (71)	0.39	0.074	0.011	0.96	0.14	0.019	0.94	0.54	0.13	0.82	0.40	0.11
9	1555835	rs771924	*DMRT2/ SMARCA2*	FVC	7.2×10^{-9} (71)	0.080	0.47	9.1×10^{-3}	0.19	0.87	0.012	0.046	0.36	7.6×10^{-3}	0.22	0.77	0.016

9	1574877	rs9407640		FVC, FEV_1	2.9×10^{-8} (71)	0.21	0.72	0.033	0.55	0.43	0.027	0.24	0.68	0.092	0.76	0.39	0.080
9	139094805	rs2274116	*LHX3*	FVC	5.5×10^{-14} (70)	0.42	0.70	3.5×10^{-3}	0.31	0.42	3.0×10^{-3}	0.59	0.46	2.9×10^{-3}	0.27	0.47	3.2×10^{-3}
9	139257411	rs10870202	*DNLZ*	FVC	9.3×10^{-10} (75)	0.78	0.83	0.95	0.83	0.88	0.98	0.32	0.20	0.36	1.00	0.72	0.78
10	69957350	rs7095607	*MYPN*	FVC	8.7×10^{-15} (75)	0.77	0.54	0.58	0.83	0.36	0.31	0.71	0.56	0.75	0.67	0.35	0.47
10	77002679	10:77002679:TC_T	*COMTD1/ ZNF503-AS1*	FVC	4.9×10^{-8} (71)	0.011	0.050	0.023	0.014	0.079	0.039	0.29	0.61	0.26	0.13	0.40	0.22
11	43648368	rs4237643	*HSD17B12*	FVC	3.5×10^{-8} (77)	0.33	0.66	0.28	0.80	0.64	0.36	0.11	0.53	0.021	0.66	0.34	0.028
11	45250732	rs2863171	*PRDM11/ SYT13*	FVC	9.0×10^{-10} (77)	0.34	0.92	0.051	0.064	0.43	0.057	0.014	0.14	3.1×10^{-3}	2.5×10^{-3}	0.092	2.2×10^{-3}
11	127995904	rs73025192	*KIRREL3-AS3/ETS1*	FVC	1.6×10^{-8} (71)	0.47	0.35	0.58	0.73	0.58	0.83	0.54	0.64	0.79	0.66	0.86	0.85
12	28283187	rs11383346	*CCDC91*	FVC	9.5×10^{-18} (70)	0.62	0.91	0.23	0.93	0.42	0.16	0.18	0.55	0.069	0.34	0.98	0.10
12	85724305	rs7971039	*ALX1/ RASSF9*	FVC, FEV_1	1.4×10^{-8} (71)	0.79	0.70	0.23	0.95	0.41	0.23	0.15	0.63	0.034	0.71	0.25	0.021
12	85724096	rs10779158		FVC	1.5×10^{-8} (71)	0.48	0.96	0.18	0.90	0.35	0.13	0.31	0.95	0.019	0.84	0.16	0.011
12	94184082	rs11107184	*CRADD*	FVC	3.9×10^{-8} (71)	0.14	0.075	0.18	0.27	0.12	0.26	0.29	0.14	0.24	0.30	0.082	0.14
12	94852628	rs10859698	*CCDC41*	FVC	3.5×10^{-8} (71)	0.67	0.34	0.27	0.34	0.75	0.37	0.92	0.49	0.29	0.41	0.91	0.32
12	115500691	rs35506	*TBX3/ MED13L*	FVC	9.9×10^{-10} (75)	0.16	0.32	0.27	0.38	0.83	0.40	0.058	0.17	0.092	0.27	0.80	0.22
15	46722435	rs4775429	*SQRDL/ SEMA6D*	FVC	2.5×10^{-8} (71)	0.14	0.039	0.027	0.86	0.58	0.26	0.16	0.078	0.12	0.41	0.29	0.40
15	67483276	rs8025774	*SMAD3*	FVC	9.3×10^{-13} (71)	0.45	0.88	0.24	0.56	0.72	0.19	0.47	0.85	0.047	0.29	0.79	0.035
16	70040398	rs3973397	*PDXDC2P*	FVC	3.3×10^{-8} (71)	0.031	0.074	0.078	0.60	0.83	0.32	1.9×10^{-3}	0.011	4.3×10^{-3}	0.40	0.68	0.088

16	72252097	rs55771535	*PMFBP1/ ZFHX3*	FVC	6.4×10^{-10} (71)	0.031	0.049	0.096	9.0×10^{-3}	8.7×10^{-3}	0.25	0.020	0.15	7.3×10^{-3}	1.5×10^{-3}	0.034	3.3×10^{-3}
16	78187138	rs1079572	*WWOX*	FVC	1.0×10^{-8} (77)	0.49	0.68	9.9×10^{-3}	0.17	0.94	0.015	0.11	0.48	0.028	0.16	0.95	0.031
17	37611352	rs8067511	*MED1/ CDK12*	FVC	1.1×10^{-8} (71)	0.11	0.29	0.14	0.063	0.31	0.090	0.055	0.15	0.10	0.023	0.13	0.050
17	43682405	rs150741403	*LOC644172/ CRHR1*	FVC	1.9×10^{-9} (71)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
17	68976415	rs6501431	*KCNJ2*	FVC	2.9×10^{-9} (77)	1.00	0.82	0.82	0.15	0.19	0.36	0.41	0.16	0.17	0.17	0.47	0.27
18	20728158	rs7238093	*CABLES1*	FVC	6.8×10^{-9} (71)	0.31	0.78	2.0×10^{-3}	0.031	0.58	3.4×10^{-3}	0.14	0.74	4.9×10^{-3}	4.0×10^{-3}	0.15	2.2×10^{-3}
18	50957922	rs8089865	*DCC*	FVC	2.0×10^{-10} (71)	0.86	0.96	0.94	0.85	0.99	0.92	0.42	0.83	0.24	0.89	0.42	0.18
20	6632901	rs6140050	*CASC20/ BMP2*	FVC	6.4×10^{-14} (75)	7.0×10^{-3}	0.016	0.025	5.6×10^{-3}	0.018	0.021	0.010	0.064	0.013	6.2×10^{-3}	0.086	0.011
20	45529571	rs2236519	*EYA2*	FVC	3.5×10^{-8} (71)	0.87	0.47	0.28	0.57	0.92	0.42	0.84	0.35	0.13	0.42	0.92	0.22
1	17306675	rs2284746	*MFAP2*	FEV_1/FVC	7.5×10^{-16} (73)	0.32	0.61	0.34	0.099	0.33	0.19	0.10	0.34	0.085	0.061	0.43	0.058
1	40035686	rs17513135	*LOC 101929516*	FEV_1/FVC	2.3×10^{-16} (75)	0.18	0.61	0.057	9.0×10^{-3}	0.11	0.014	0.38	0.54	0.59	0.094	0.17	0.24
1	92068967	rs1192404	*CDC7/ TGFBR3*	FEV_1/FVC	6.1×10^{-20} (75)	0.60	0.26	0.20	0.79	0.29	0.26	0.91	0.38	0.041	0.62	0.43	0.053
1	92374517	rs12140637	*TGFBR3/ BRDT2*	FEV_1/FVC	1.2×10^{-9} (75)	0.45	0.58	0.68	0.61	0.94	0.68	0.69	0.38	0.35	0.76	0.30	0.30
1	160206067	rs11591179	*DCAF8*	FEV_1/FVC	3.5×10^{-8} (71)	0.51	0.85	0.41	1.00	0.45	0.31	0.87	0.68	0.28	0.76	0.54	0.22
1	218860068	rs993925	*TGFB2*	FEV_1/FVC	1.2×10^{-8} (73)	0.56	0.53	5.4×10^{-3}	0.89	0.046	1.1×10^{-3}	0.29	0.93	0.024	0.60	0.35	0.021
1	219963090	rs201204531	*LYPLAL1/ RNUSF-1*	FEV_1/FVC	2.7×10^{-10} (70)	0.40	0.52	0.66	0.65	0.79	0.88	0.96	0.91	0.92	0.44	0.52	0.75
1	239850588	rs6688537	*CHRM3*	FEV_1/FVC	6.7×10^{-22} (75)	0.52	0.52	0.81	0.26	0.19	0.43	0.20	0.13	0.30	0.12	0.05	0.14

2	10418806	rs139215025	*C2orf48/ HPCAL1*	FEV_1/FVC	9.0×10^{-11} (71)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	18292452	rs61067109	*KCNS3/ RDH14*	FEV_1/FVC	1.4×10^{-15} (70)	0.32	0.53	0.45	0.50	0.99	0.45	0.34	0.49	0.55	0.59	0.97	0.63
2	157046432	rs72904209	*KCNJ3/ NR4A2*	FEV_1/FVC	3.1×10^{-8} (71)	0.78	0.82	0.39	0.84	0.32	0.25	0.33	0.12	0.13	0.72	0.30	0.33
2	229502503	rs10498230	*PID1*	FEV_1/FVC	3.9×10^{-8} (9)	0.43	1.00	0.097	0.26	0.88	0.048	0.41	0.47	0.71	0.48	0.66	0.74
2	229510929	rs1435867	*PID1*	FEV_1/FVC	3.7×10^{-8} (9)	0.45	0.95	0.085	0.27	0.84	0.042	0.42	0.48	0.72	0.52	0.71	0.77
2	230224031	rs7594321	*DNER*	FEV_1/FVC	2.6×10^{-9} (4)	0.15	0.67	0.022	0.61	0.31	0.012	0.44	0.57	0.69	0.70	0.89	0.87
2	239316560	rs61332075	*TRAF3IP1/ ASB1*	FEV_1/FVC	2.6×10^{-10} (75)	0.83	0.63	0.73	0.24	0.32	0.51	0.27	0.31	0.54	0.54	0.35	0.60
2	239877148	rs12477314	*FLJ43879/ HDAC4*	FEV_1/FVC	1.7×10^{-12} (73)	0.35	0.94	0.020	0.56	0.28	5.8×10^{-3}	0.24	0.60	0.14	0.31	0.96	0.15
3	25520582	rs1529672	*RARB*	FEV_1/FVC	4.0×10^{-14} (73)	0.26	0.75	2.9×10^{-4}	0.017	0.53	5.5×10^{-4}	0.73	0.26	0.090	0.046	0.19	0.097
3	29431565	rs28723417	*RBMS3*	FEV_1/FVC	1.8×10^{-8} (71)	0.76	0.78	0.24	0.38	0.99	0.22	0.59	0.54	0.82	0.71	0.62	0.88
3	29469675	rs17666332		FEV_1/FVC	4.8×10^{-8} (71)	0.43	0.76	0.32	0.22	0.63	0.23	0.98	0.99	0.99	0.90	0.95	0.98
3	55150677	rs1458979	*CACNA2D3/ WNT5A*	FEV_1/FVC	4.4×10^{-10} (75)	0.11	0.19	0.26	0.17	0.28	0.37	0.44	0.30	0.50	0.43	0.16	0.27
3	62386350	rs111793843	*CADPS*	FEV_1/FVC	2.0×10^{-8} (71)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	99359368	rs80217917	*DCBLD2/ MIR548G*	FEV_1/FVC	2.6×10^{-8} (71)	0.81	0.50	0.43	0.27	0.50	0.46	0.32	0.19	0.35	0.55	0.77	0.77
3	127991527	rs2811415	*EEFSEC*	FEV_1/FVC	5.5×10^{-11} (75)	0.73	0.50	0.56	0.58	0.85	0.71	0.87	0.74	0.86	0.68	0.84	0.88
3	168715808	rs56341938	*LOC100507661/MECOM*	FEV_1/FVC	4.5×10^{-14} (75)	0.49	0.51	0.79	0.13	0.13	0.29	0.17	0.12	0.28	0.47	0.38	0.67
4	7846240	rs28520091	*AFAP1*	FEV_1/FVC	2.2×10^{-9} (71)	0.33	0.33	0.61	0.15	0.82	0.22	0.49	0.44	0.74	0.40	0.26	0.51

4	89777081	rs6830970	*FAM13A*	FEV_1/FVC	1.9×10^{-8} (9)	0.59	0.92	0.19	0.056	0.24	0.10	0.26	0.41	0.44	0.88	0.48	0.52
4	89815695	rs13110699	*FAM13A*	FEV_1/FVC	7.9×10^{-15} (75)	0.79	0.64	0.82	0.92	0.86	0.88	0.30	0.66	0.22	0.26	0.79	0.21
4	89869332	rs2869967	*FAM13A*	FEV_1/FVC	1.6×10^{-8} (9)	0.44	0.71	0.44	0.049	0.15	0.13	0.35	0.65	0.32	0.72	0.24	0.23
4	106841962	rs6856422	*NPNT*	FEV_1/FVC	1.5×10^{-23} (70)	0.33	0.96	0.050	0.093	0.51	0.065	0.87	0.65	0.25	0.17	0.53	0.22
4	145434584	rs1032295	*HHIP*	FEV_1/FVC	4.4×10^{-15} (9)	0.68	0.71	0.11	0.47	0.73	0.12	0.92	0.90	0.99	0.73	0.71	0.93
4	145436324	rs12504628	*HHIP*	FEV_1/FVC	6.5×10^{-13} (72)	0.64	0.53	0.018	0.35	0.63	0.023	0.58	0.62	0.86	0.85	0.93	0.97
4	145485738	rs1980057	*HHIP*	FEV_1/FVC	3.2×10^{-20} (9)	0.34	0.87	0.013	0.29	0.64	0.014	0.92	0.92	0.99	0.97	0.93	1.00
5	52195033	rs1551943	*ITGA1*	FEV_1/FVC	1.9×10^{-18} (75)	0.12	0.40	0.075	0.27	0.93	0.13	0.95	0.98	0.99	1.00	0.99	1.00
5	95036700	rs153916	*SPATA9*	FEV_1/FVC	2.1×10^{-8} (73)	0.19	0.24	0.042	0.12	0.15	0.29	0.52	0.35	0.54	0.96	0.77	0.80
5	131788334	rs7713065	*C5orf56*	FEV_1/FVC	2.8×10^{-11} (75)	0.34	0.86	0.085	0.012	0.086	0.028	0.66	0.55	0.79	0.19	0.18	0.38
5	147842353	rs11168048	*HTR4*	FEV_1/FVC	1.1×10^{-11} (9)	1.1×10^{-3}	9.9×10^{-3}	2.2×10^{-3}	0.062	0.64	0.016	0.035	0.016	0.052	0.63	0.55	0.84
5	147844392	rs7735184	*HTR4*	FEV_1/FVC	6.2×10^{-11} (9)	7.8×10^{-4}	6.4×10^{-3}	1.7×10^{-3}	0.051	0.57	0.015	0.026	0.011	0.039	0.56	0.50	0.79
5	156810072	rs10515750	*CYFIP2*	FEV_1/FVC	5.3×10^{-13} (75)	0.98	0.48	0.15	0.32	0.94	0.20	0.61	0.77	0.13	0.15	0.60	0.15
5	156932376	rs2277027	*ADAM19*	FEV_1/FVC	9.9×10^{-11} (9)	0.87	0.29	4.2×10^{-3}	0.51	0.30	5.0×10^{-3}	0.59	0.56	0.85	0.56	0.53	0.82
5	156936364	rs1422795	*ADAM19*	FEV_1/FVC	2.6×10^{-10} (9)	0.90	0.26	3.3×10^{-3}	0.51	0.29	4.1×10^{-3}	0.56	0.52	0.81	0.54	0.51	0.80
6	22017738	rs1928168	*LINC00340*	FEV_1/FVC	6.7×10^{-14} (71)	0.063	0.24	0.058	0.11	0.54	0.091	0.42	0.53	0.69	0.064	0.070	0.16
6	22021373	rs9350408		FEV_1/FVC	7.5×10^{-14} (71)	0.25	0.46	0.37	0.52	0.99	0.48	0.42	0.34	0.63	0.20	0.11	0.27

6	31556155	rs28986170	*LST1*	FEV_1/FVC	1.6×10^{-10} (75)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6	31568469	rs2857595	*NCR3*	FEV_1/FVC	2.3×10^{-10} (73)	0.067	0.049	0.14	0.21	0.14	0.34	0.25	0.090	0.14	0.54	0.78	0.74
6	32124424	rs10947233	*PPT2*	FEV_1/FVC	6.7×10^{-12} (9)	0.99	0.86	0.93	0.34	0.18	0.39	0.18	0.74	0.047	0.18	0.82	0.093
6	32151443	rs2070600	*AGER*	FEV_1/FVC	3.1×10^{-15} (9, 72)	0.56	0.65	0.83	0.34	0.14	0.29	0.38	0.89	0.052	0.14	0.73	0.074
6	32680576	rs7764819	*HLA-DQB1/ HLA-DQA2*	FEV_1/FVC	4.4×10^{-9} (4)	0.68	0.28	0.15	0.27	0.71	0.26	0.85	0.92	0.97	0.28	0.22	0.48
6	67863782	rs9351637	*SLC25A51P1 /BAI3*	FEV_1/FVC	2.9×10^{-8} (71)	0.54	0.78	0.59	0.90	0.74	0.67	0.20	0.76	0.027	0.23	0.90	0.036
6	73670095	rs141651520	*KCNQ5*	FEV_1/FVC	9.9×10^{-18} (75)	0.89	0.86	0.67	0.76	0.85	0.81	0.45	0.40	0.70	0.89	0.92	0.97
6	109268050	rs2798641	*ARMC2*	FEV_1/FVC	8.4×10^{-9} (73)	0.22	0.44	0.28	0.19	0.41	0.34	0.97	0.58	0.39	0.55	0.97	0.54
6	142691549	rs11155242	*GPR126*	FEV_1/FVC	9.1×10^{-9} (9)	0.061	0.10	0.17	0.10	0.18	0.26	0.26	0.21	0.45	0.34	0.26	0.53
6	142707133	rs6937121	*GPR126*	FEV_1/FVC	2.5×10^{-9} (9)	0.37	0.75	0.27	0.30	0.69	0.35	0.49	0.50	0.78	0.95	0.81	0.93
6	142750516	rs3817928	*GPR126*	FEV_1/FVC	2.6×10^{-10} (9)	0.13	0.23	0.27	0.088	0.18	0.23	0.37	0.32	0.61	0.24	0.16	0.38
6	142777064	rs7776375	*GPR126/ HIVEP2*	FEV_1/FVC	1.3×10^{-9} (9)	0.40	0.69	0.42	0.41	0.74	0.55	0.36	0.39	0.66	0.99	0.78	0.88
6	142838173	rs148274477	*GPR126*	FEV_1/FVC	9.6×10^{-26} (70)	0.30	0.29	0.56	0.81	0.84	0.96	0.091	0.17	0.20	0.16	0.25	0.22
7	7286445	rs10246303	*C1GALT1*	FEV_1/FVC	2.4×10^{-8} (75)	0.055	0.075	0.16	0.67	0.95	0.65	0.027	0.022	0.071	0.24	0.23	0.47
7	99635967	rs72615157	*ZKSCAN1*	FEV_1/FVC	2.0×10^{-9} (75)	0.72	0.81	0.90	0.45	0.24	0.43	0.86	0.76	0.90	0.42	0.42	0.71
7	146651409	rs1404154	*CNTNAP2*	FEV_1/FVC	2.8×10^{-8} (71)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
9	23588583	rs10965947	*FLJ35282/ ELAVL2*	FEV_1/FVC	2.7×10^{-9} (71)	0.18	0.51	0.13	0.080	0.44	0.078	0.78	0.75	0.28	0.23	0.67	0.23

9	98231008	rs16909898	*PTCH1*	FEV_1/FVC	1.8×10^{-8} (9)	0.32	0.12	0.15	0.87	0.42	0.43	0.96	0.39	0.059	0.79	0.092	0.022
9	98256309	rs10512249	*PTCH1*	FEV_1/FVC	2.8×10^{-8} (9)	0.35	0.19	0.32	0.51	0.26	0.45	0.71	0.66	0.11	0.73	0.096	0.033
9	109496630	rs2451951	*TMEM38B/ ZNF462*	FEV_1/FVC	2.4×10^{-8} (71)	0.30	0.40	0.56	0.34	0.49	0.61	0.30	0.17	0.31	0.38	0.16	0.30
9	119359372	rs34886460	*ASTN2*	FEV_1/FVC	4.7×10^{-11} (70)	0.17	0.29	0.30	0.92	0.61	0.43	0.88	0.66	0.68	0.67	0.94	0.70
10	12277992	rs7068966	*CDC123*	FEV_1/FVC	6.1×10^{-13} (73)	0.13	0.68	6.6×10^{-3}	0.10	0.96	6.0×10^{-3}	0.72	1.00	0.62	0.95	0.64	0.59
10	30267810	rs3847402	*SVIL/ KIAA1462*	FEV_1/FVC	7.7×10^{-11} (75)	0.39	0.43	0.69	0.33	0.41	0.62	0.47	0.73	0.51	0.26	0.53	0.43
10	64916064	rs75159994	*JMJD1C*	FEV_1/FVC	6.1×10^{-9} (71)	0.067	0.35	0.015	0.059	0.62	0.011	0.17	0.70	0.019	0.050	0.54	0.016
10	124273671	rs2293871	*HTRA1*	FEV_1/FVC	1.5×10^{-8} (71)	0.54	0.32	0.44	0.51	0.19	0.28	0.36	0.34	0.63	0.37	0.31	0.59
11	73280955	11:73280955: GA_G	*FAM168A*	FEV_1/FVC	2.7×10^{-8} (71)	0.10	0.11	0.26	0.84	0.45	0.52	0.12	0.19	0.28	0.98	0.58	0.53
12	57527283	rs11172113	*LRP1*	FEV_1/FVC	1.2×10^{-8} (73)	4.1×10^{-3}	0.030	6.7×10^{-3}	0.60	0.39	0.033	0.047	0.18	0.054	0.40	0.86	0.15
12	95554771	rs113745635	*FGD6*	FEV_1/FVC	8.5×10^{-18} (75)	0.91	0.62	0.25	0.25	0.70	0.25	0.82	0.96	0.77	0.11	0.15	0.27
12	96271428	rs1036429	*CCDC38*	FEV_1/FVC	2.3×10^{-11} (73)	0.44	0.35	0.62	0.57	0.42	0.70	0.65	0.71	0.099	0.92	0.26	0.067
14	54410919	rs4444235	*DDHD1\ MIR5580*	FEV_1/FVC	4.0×10^{-8} (71)	0.18	0.61	0.065	0.042	0.32	0.050	0.52	0.85	0.47	0.068	0.14	0.19
14	84309664	rs1698268	*LINC01467/LINC00911*	FEV_1/FVC	3.2×10^{-8} (75)	0.80	0.85	0.96	0.44	0.24	0.44	0.73	0.80	0.92	0.62	0.35	0.54
15	41977690	rs72724130	*MGA*	FEV_1/FVC	9.6×10^{-10} (75)	0.45	0.36	0.63	0.30	0.14	0.30	0.81	0.81	0.44	0.54	0.15	0.16
15	50555681	rs180930492	*HDC*	FEV_1/FVC	2.6×10^{-9} (71)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
15	71645120	rs12899618	*THSD4*	FEV_1/FVC	7.2×10^{-15} (72)	0.20	0.53	0.14	0.52	0.76	0.19	0.73	0.87	0.86	0.99	0.87	0.95

15	71788387	rs12591467	*THSD4*	FEV_1/FVC	5.7×10^{-10} (75)	0.97	0.87	0.87	0.24	0.28	0.49	0.32	0.17	0.31	0.15	0.034	0.079
15	84261689	rs66650179	*SH3GL3*	FEV_1/FVC	3.7×10^{-12} (75)	0.22	0.27	0.46	0.46	0.56	0.65	0.26	0.27	0.52	0.049	0.050	0.14
16	10706328	rs12149828	*EMP2/ TEKT5*	FEV_1/FVC	7.7×10^{-10} (70)	9.6×10^{-3}	0.023	0.031	0.16	0.44	0.26	0.058	0.038	0.12	0.37	0.30	0.59
16	58075282	rs12447804	*MMP15*	FEV_1/FVC	3.6×10^{-8} (73)	0.052	0.17	0.078	0.13	0.48	0.16	0.99	0.73	0.66	0.37	0.17	0.35
16	66060569	rs144296676	*LOC283867/ CDH5*	FEV_1/FVC	5.4×10^{-9} (71)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
16	75390316	rs2865531	*CFDP1*	FEV_1/FVC	1.8×10^{-11} (73)	0.34	0.98	0.025	0.30	0.78	0.039	0.53	0.80	0.56	0.56	0.91	0.62
17	28263980	rs62070270	*EFCAB5*	FEV_1/FVC	7.3×10^{-18} (75)	0.28	0.34	0.56	0.90	0.65	0.78	0.58	0.80	0.69	0.99	0.61	0.60
17	36886828	rs11658500	*CISD3*	FEV_1/FVC	7.2×10^{-11} (75)	0.034	0.12	0.054	0.01	0.04	0.02	0.13	0.20	0.31	0.084	0.12	0.22
19	31829613	rs9636166	*TSHZ3*	FEV_1/FVC	3.3×10^{-9} (71)	0.58	0.74	0.78	0.099	0.13	0.25	0.60	0.51	0.79	0.25	0.17	0.39
19	31846907	rs1353531		FEV_1/FVC	4.5×10^{-8} (71)	0.48	0.87	0.34	0.029	0.10	0.079	0.47	0.71	0.55	0.029	0.057	0.091
19	41124155	rs113473882	*LTBP4*	FEV_1/FVC	1.0×10^{-12} (70)	0.72	0.59	0.79	0.80	0.77	0.96	0.85	0.49	0.24	0.70	0.92	0.61
19	50213396	rs147472287	*CPT1C*	FEV_1/FVC	3.3×10^{-8} (71)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
21	35652239	rs9978142	*MRPS6/ KCNE2*	FEV_1/FVC	2.7×10^{-8} (73)	0.56	0.32	0.39	0.83	0.51	0.61	0.082	0.10	0.22	0.46	0.70	0.67
22	20854161	rs4820216	*KLHL22/ MED15*	FEV_1/FVC	2.6×10^{-8} (71)	4.1×10^{-3}	0.015	0.013	0.019	0.071	0.060	0.048	0.057	0.14	0.11	0.12	0.27
X	15964845	rs7050036	*APIS2/GRPR*	FEV_1/FVC	4.1×10^{-8} (70)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Abbreviations: chr = Chromosome; DHA = Docosahexaenoic acid; DPA = Docosapentaenoic acid; FEV_1 = Forced expiratory volume in the first second; FVC = Forced vital capacity; GWAS = Genome-wide association study; int = Interaction; NA = Not available; SNP = Single nucleotide polymorphism.

*SNPs with the smallest P values from each locus reported in the prior GWAS analysis are shown and are sorted by the originally indicated PFT measure and then by chromosomal position (NCBI build 37). SNPs that passed the multiple testing correction (*n* of tests = 199, Bonferroni-corrected $P<2.5\times10^{-4}$) are bolded.

†Originally reported SNP rs114229351 has merged into rs9275068.

Table E12. HaploReg v4.1 functional annotation of rs11693320 and variants with $r^2 > 0.8$ in the 1000 Genomes European (EUR) reference panel*

Chr. 2 position (hg38)	LD (r^2)	LD (D')	Variant	Reference allele	Alternative allele	AFR freq	EUR freq	Enhancer histone marks	Motifs changed	dbSNP functional annotation
114547473	0.8	0.93	rs7581096	C	G	0.59	0.18	GI	HDAC2, Ik-2, NF-AT1, NF-I, Pou2f2, Pou3f3, TATA	intronic
114547629	0.8	0.93	rs7606971	G	T	0.6	0.18	GI	Foxf1, Foxi1, Foxp1	intronic
114548134	0.85	0.98	rs1430112	A	G	0.49	0.19	GI, PANC	ATF2, CEBPA, CEBPB, E4BP4, Evi-1, Rad21	intronic
114548855	0.85	0.98	rs6738538	C	T	0.54	0.19	ESC, GI, PANC		intronic
114549091	0.85	0.98	rs2033305	G	A	0.45	0.81	ESC, IPSC, GI, PANC, LNG, LIV	BDP1, GR, Ik-1, Ik-2, Ik-3, Pax-4	intronic
114549684	0.85	0.98	rs201802986	A	AG	0.17	0.19	BLD, GI, LNG, LIV		intronic
114549695	0.83	0.98	rs66601344	GAAAT	G	0.45	0.81	BLD, GI, LNG, LIV	Dbx1, Evi-1, Foxd3, Foxi1, Foxj2, Foxk1, Foxl1, Foxo, Foxp1, HDAC2, Hoxb9, Irf, Nkx6-2, Pou1f1, TATA, Zfp105, p300	intronic
114550803	0.83	0.95	rs13008052	G	A	0.16	0.18	GI	FXR, GR	intronic
114552197	0.84	0.96	rs4289198	G	A	0.15	0.18	LIV	Irf	intronic
114552344	0.84	0.96	rs5833551	GT	G	0.45	0.82		DBP, Smad3	intronic
114552481	0.84	0.96	rs2082131	C	T	0.35	0.18		Cdc5	intronic
114553326	0.84	0.96	rs11886546	A	G	0.15	0.18		HNF1, Ncx	intronic
114557050	0.86	0.98	rs12711799	G	A	0.45	0.81		HMG-IY	intronic
114562002	0.86	0.98	rs1864437	A	G	0.58	0.19			intronic
114562416	0.86	0.98	rs1367183	G	A	0.54	0.19		Arid3a, Dbx1, HP1-site-factor, Lhx3, Ncx, Pou2f2, Pou3f4, Pou5f1, Sox, TATA	intronic
114563156	0.86	0.98	rs1835330	A	G	0.59	0.19		FXR, HDAC2, HNF4, NR4A, RAR, RXRA	intronic
114566124	0.86	0.98	rs35463802	G	C	0.21	0.19	GI	HDAC2, Pou6f1, Spz1	intronic
114567030	0.86	0.98	rs11683255	T	C	0.28	0.19	ESDR	ZBTB33	intronic

114568698	0.85	0.97	rs6746835	G	A	0.58	0.19	BRN	Foxa, Foxd1, Foxf2, Foxi1, HDAC2, Homez, TCF12, p300	intronic
114573143	1	1	rs6735899	C	T	0.39	0.83		ERalpha-a, NRSF	intronic
114574293	1	1	rs11693320	A	G	0.21	0.17		Glis2	intronic
114574534	1	1	rs11694667	T	G	0.18	0.17		HNF1, PLZF	intronic
114576974	0.98	1	rs1835329	C	T	0.59	0.17		Isl2	intronic
114579673	0.96	0.99	rs10496466	G	A	0.14	0.17			intronic

Abbreviations: AFR = African populations from 1000 Genomes; BLD = Blood tissue; BRN = Brain tissue; chr = Chromosome; dbSNP = Single Nucleotide Polymorphism database; ESC = Embryonic stem cells; ESDR = ESC-derived cells; EUR = European populations from 1000 Genomes; freq = Frequency; GENCODE = Reference human genome annotation for the ENCODE project; GI = Gastrointestinal tissue; IPSC = Induced pluripotent stem cells; LD = Linkage disequilibrium; LIV = Liver tissue; LNG = Lung tissue; PANC = Pancreas tissue.

*All variants are annotated to the *DPP10* gene.

Table E13. *DPP10* SNPs implicated at $P_{2df} < 5\times10^{-6}$ in our cross-ancestry genome-wide joint 2df meta-analysis of FVC with DHA interaction (discovery n=11,962) and tested for association in prior HapMap-imputed GWAS analyses of FVC or DHA in cohorts of European ancestry from the CHARGE Consortium[*]

SNP and coded allele	Effect allele freq[†]	r^2 / D' with rs11693320[‡] in 1000 Genomes European (EUR) panel	r^2 / D' with rs11693320[‡] in 1000 Genomes African (AFR) panel	Joint 2df meta-analysis of FVC, accounting for DHA interaction (N=11,962)		GWAS meta-analysis of FVC (N=52,253) (77)		GWAS meta-analysis of DHA (N=8,866) (69)	
				β_{2df} direction	P_{2df}	β direction	P	β direction	P
rs1835329-C	0.78	0.99 / 1	0.17 / 0.99	-	5.0×10^{-8}	-	0.087	+	0.57
rs6746835-G	0.77	0.85 / 0.96	0.15 / 0.90	-	1.2×10^{-7}	-	0.060	+	0.55
rs1864437-A	0.77	0.85 / 0.97	0.14 / 0.88	-	1.2×10^{-7}	-	0.085	+	0.64
rs2082131-C	0.79	0.85 / 0.96	0.09 / 0.44	-	1.3×10^{-7}	-	0.100	+	0.63
rs6738538-C	0.77	0.87 / 0.99	0.04 / 0.42	-	1.4×10^{-7}	-	0.083	+	0.66
rs1367183-G	0.77	0.85 / 0.96	0.05 / 0.46	-	1.6×10^{-7}	-	0.091	+	0.55
rs11683255-T	0.80	0.88 / 0.99	0.39 / 0.82	-	1.7×10^{-7}	-	0.085	+	0.55
rs2033305-A	0.77	0.87 / 0.99	0.03 / 0.36	-	1.7×10^{-7}	-	0.080	+	0.64
rs1430112-A	0.77	0.88 / 0.99	0.01 / 0.23	-	4.9×10^{-7}	-	0.083	+	0.71
rs10496466-G	0.83	0.98 / 0.99	0.46 / 0.80	-	1.2×10^{-6}	-	0.082	+	0.71
rs7581096-C	0.76	NA[§]	NA[§]	-	1.3×10^{-6}	-	0.075	+	0.68

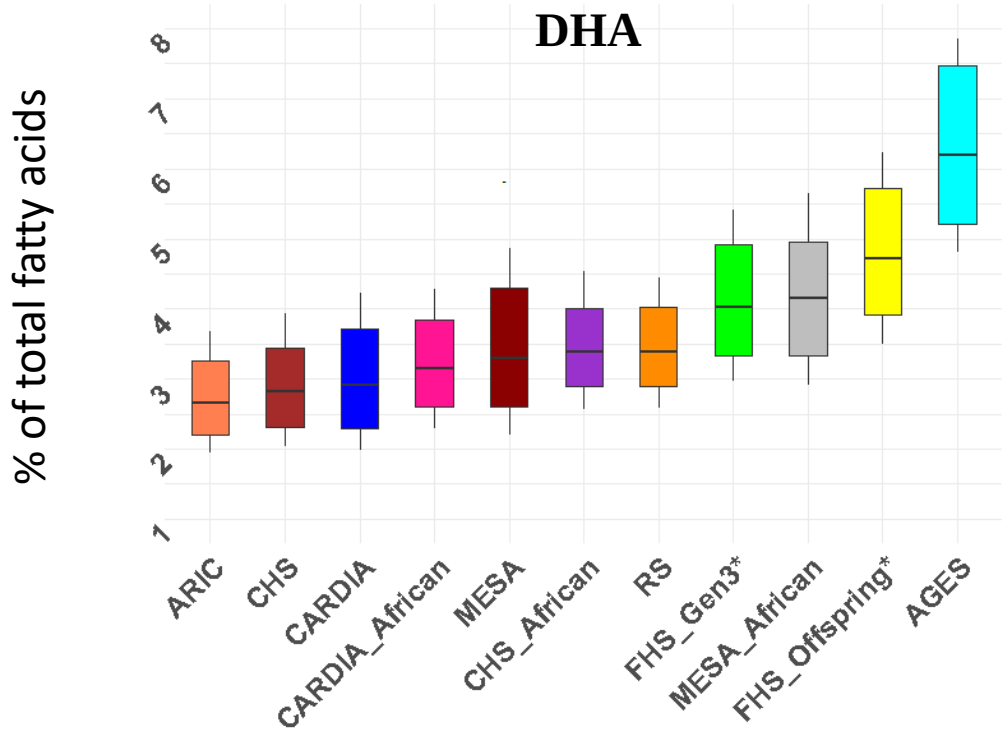
Abbreviations: DHA = Docosahexaenoic acid; freq = Frequency; FVC = Forced vital capacity; GWAS = Genome-wide association study; NA = Not available; SNP = Single nucleotide polymorphism.

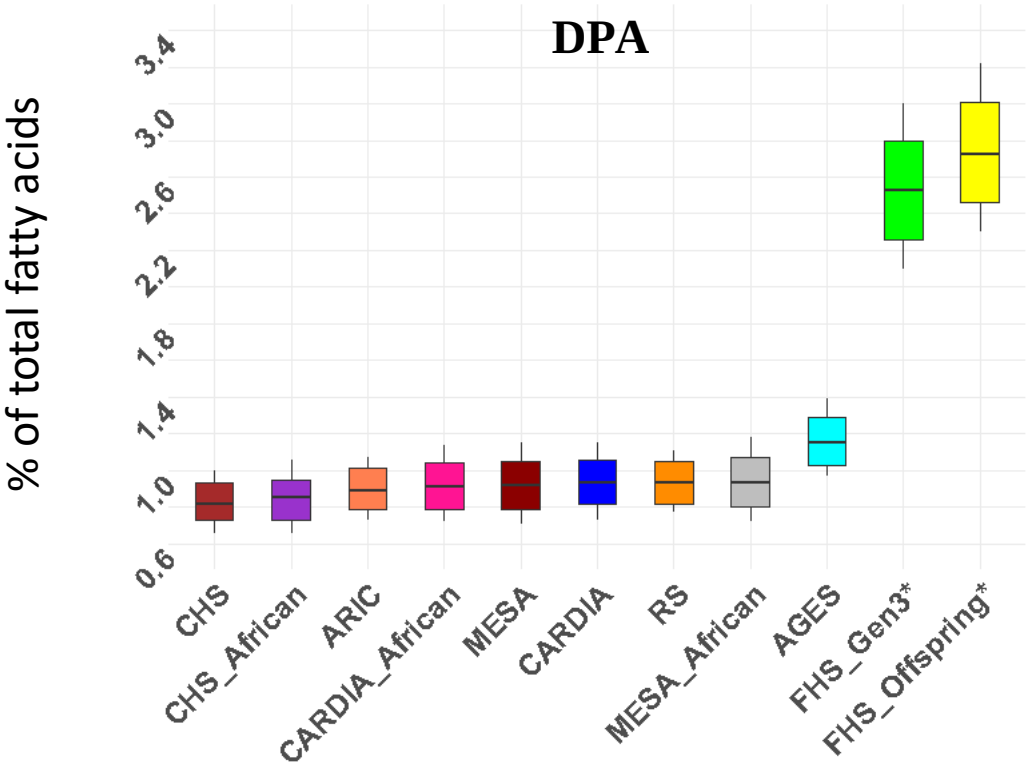
[*]The SNPs, which are all located in the same intron as the 1000 Genomes-imputed SNP rs11693320, are sorted by P_{2df} values. Linkage disequilibrium is presented with the top *DPP10* SNP rs11693320. Linkage disequilibrium estimates with rs11693320 correspond to 1000 Genomes phase 3 panels of European (EUR) or African (AFR) ancestry, as computed in LDlink (78).

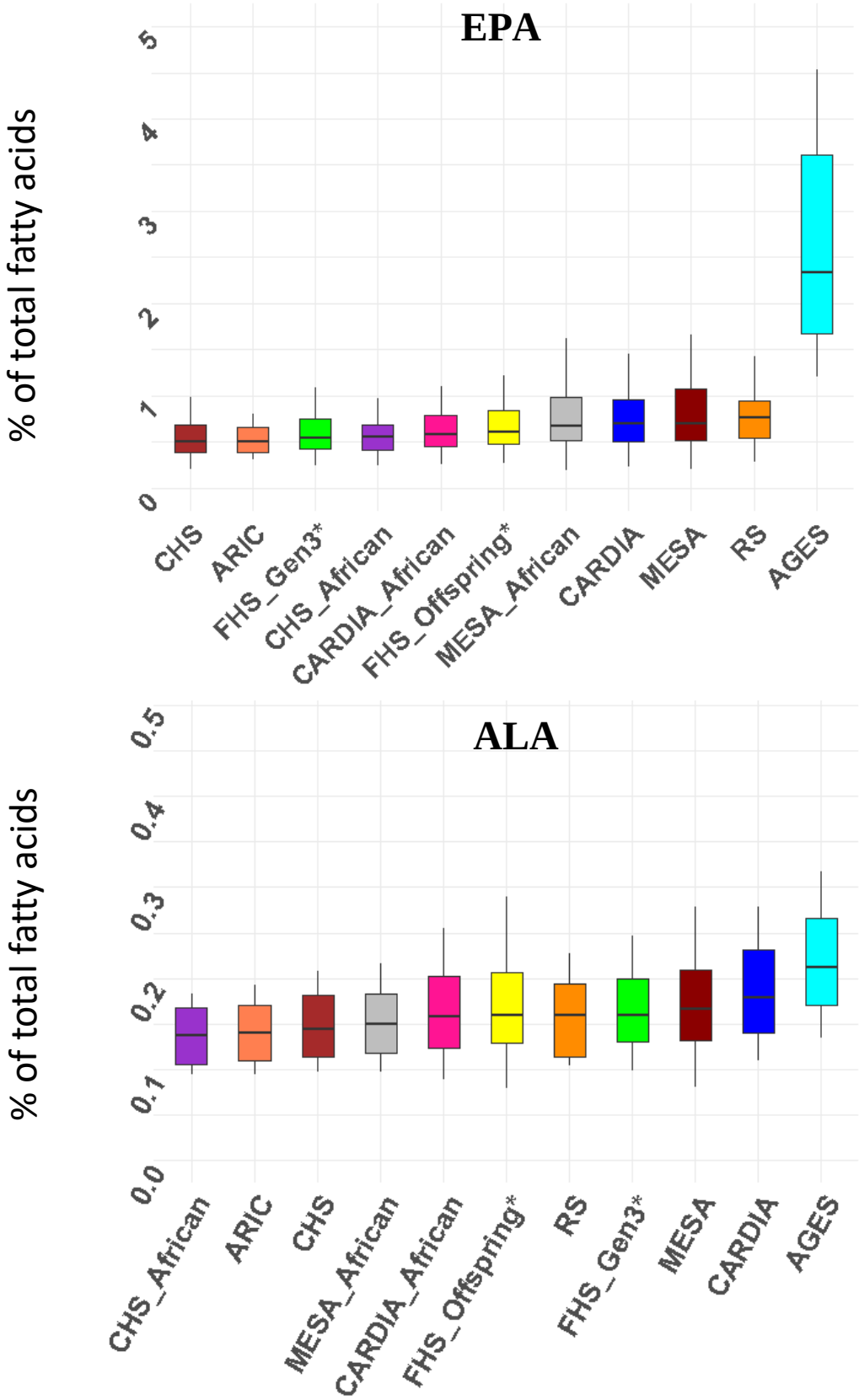
[†]Coded allele frequency weighted by sample size of cohorts in the joint 2df meta-analysis.

[‡]As a 1000 Genomes-imputed SNP, rs11693320 was not tested in the prior HapMap-based GWAS of FVC or DHA.

[§]NA, not available. Linkage disequilibrium estimates were not computed due to rs7581096 being a multi-allelic SNP.


DHA
% of total fatty acids
1
2
3
4
5
6
7
8
ARIC
CHS
CARDIA
CARDIA_African
MESA
CHS_African
RS
FHS_Gen3*
MESA_African
FHS_Offspring*
AGES


DPA
% of total fatty acids
0.6
1.0
1.4
1.8
2.2
2.6
3.0
3.4
CHS
CHS_African
ARIC
CARDIA_African
MESA
CARDIA
RS
MESA_African
AGES
FHS_Gen3*
FHS_Offspring*


EPA
% of total fatty acids
0
1
2
3
4
5
CHS
ARIC
FHS_Gen3*
CHS_African
CARDIA_African
FHS_Offspring*
MESA_African
CARDIA
MESA
RS
AGES
ALA
% of total fatty acids
0.0
0.1
0.2
0.3
0.4
0.5
CHS_African
ARIC
CHS
MESA_African
CARDIA_African
FHS_Offspring*
RS
FHS_Gen3*
MESA
CARDIA
AGES

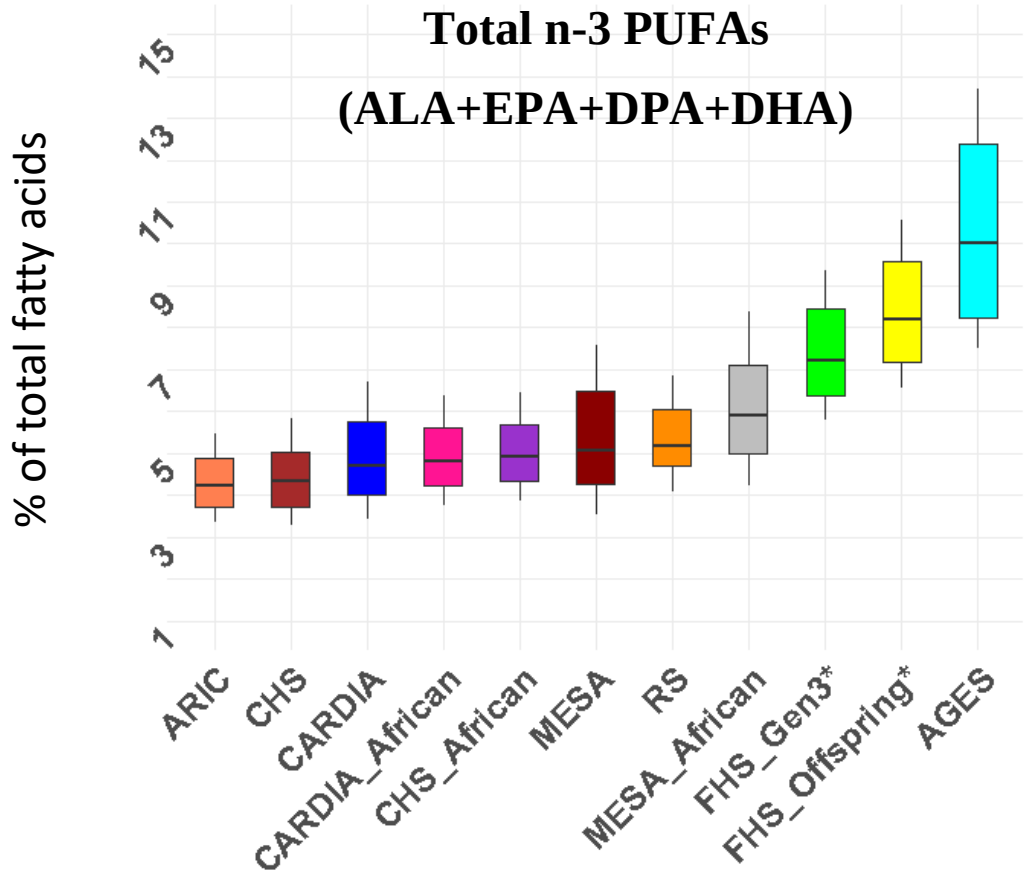


Figure E1. Distribution of omega-3 fatty acid biomarkers in each cohort. The middle bar is the median of each n-3 PUFA biomarker; the lower and upper bars of the box represent the 25th and 75th percentile values, respectively, of each n-3 PUFA biomarker; the minimum and maximum of the whisker were calculated as mean - SD, and mean + SD, respectively. Each cohort represents the European ancestry participants in that cohort, unless otherwise indicated. *FHS has omega-3 fatty acid biomarkers measured in red blood cells, rather than plasma phospholipids.

Abbreviations: AGES = Age, Gene, Environment, Susceptibility Study – Reykjavik; ALA = Alpha-linolenic acid; ARIC = Atherosclerosis Risk in Communities; CARDIA = Coronary Artery Risk Development in Young Adults; CHS = Cardiovascular Health Study; DHA = Docosahexaenoic acid; DPA = Docosapentaenoic acid; EPA = Eicosapentaenoic acid; FHS (Offspring) = Framingham Heart Study – the Offspring Cohort; FHS (Gen3) = Framingham Heart Study – the Generation 3 Cohort; MESA= Multi-Ethnic Study of Atherosclerosis; n-3 PUFA = Omega-3 poly-unsaturated fatty acid; RS = Rotterdam Study; SD = Standard deviation.

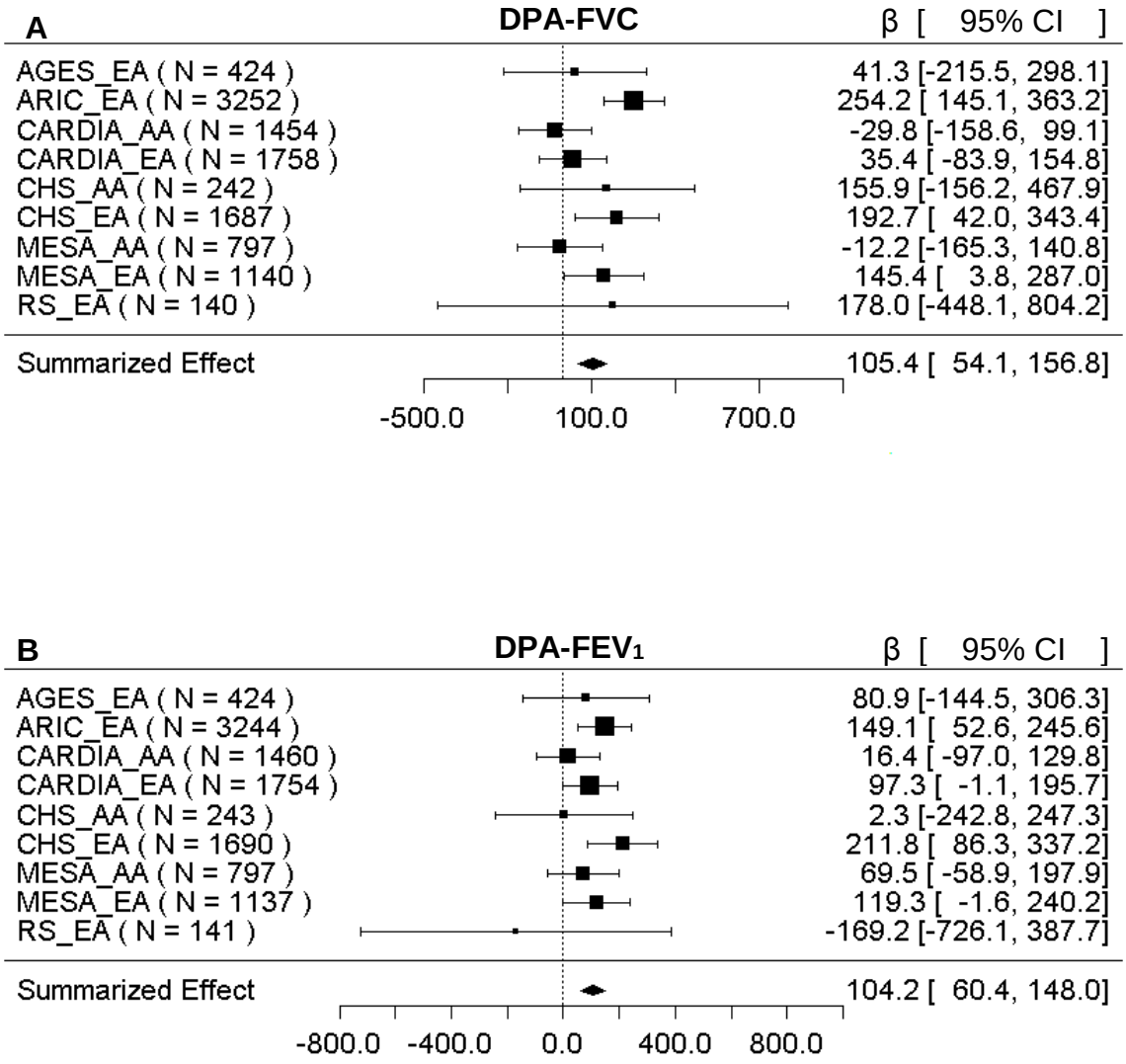


Figure E2. Forest plots of meta-analysis of the DPA plasma biomarker on pulmonary function (sensitivity analysis). Associations are presented for the DPA plasma biomarker on (A) FVC and (B) FEV_1. β (unit: mL) denotes the coefficient from the fixed-effects meta-analysis for DPA plasma biomarker on the pulmonary function test measure per 1% (of total fatty acids) increment, with its 95% confidence interval. The vertical line in the center means no effect of the DPA plasma biomarker on pulmonary function; β value to the right of the line means positive effect, while β value to the left of the line means negative effect. The size of black square for each cohort represents the variance of the β coefficient, so that cohorts with smaller

variances have larger black squares. Cohorts listed in the forest plots are ordered based on alphabetical order, with sample size of each cohort shown in the parenthesis.

Abbreviations: AA = African ancestry; AGES = Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC = Atherosclerosis Risk in Communities; CARDIA = Coronary Artery Risk Development in Young Adults; CHS = Cardiovascular Health Study; CI = Confidence interval; DPA = Docosapentaenoic acid; EA = European ancestry; FEV_1 = Forced expiratory volume in the first second; FVC = Forced vital capacity; MESA = Multi-Ethnic Study of Atherosclerosis; RS = Rotterdam (Netherlands) Study.

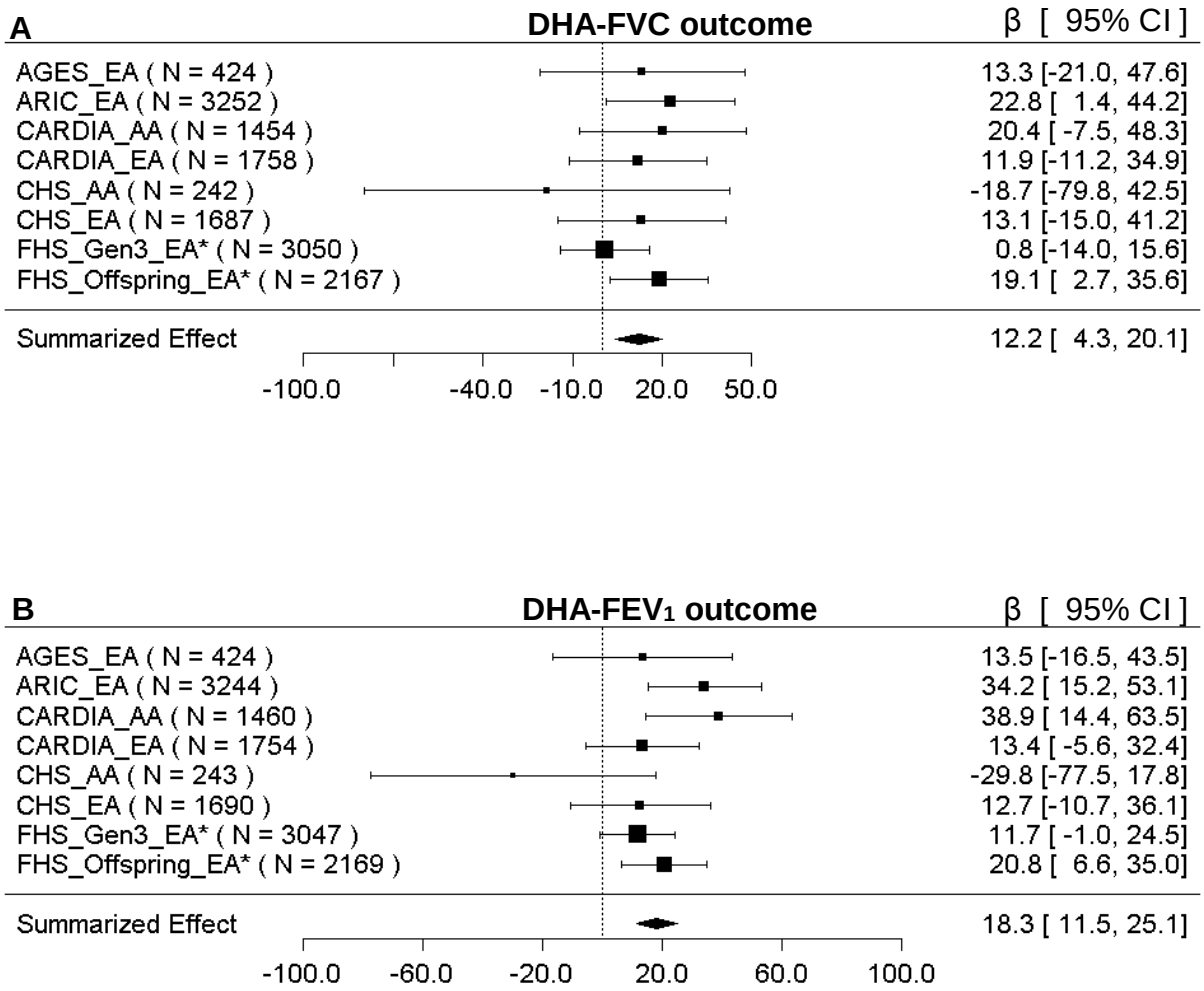
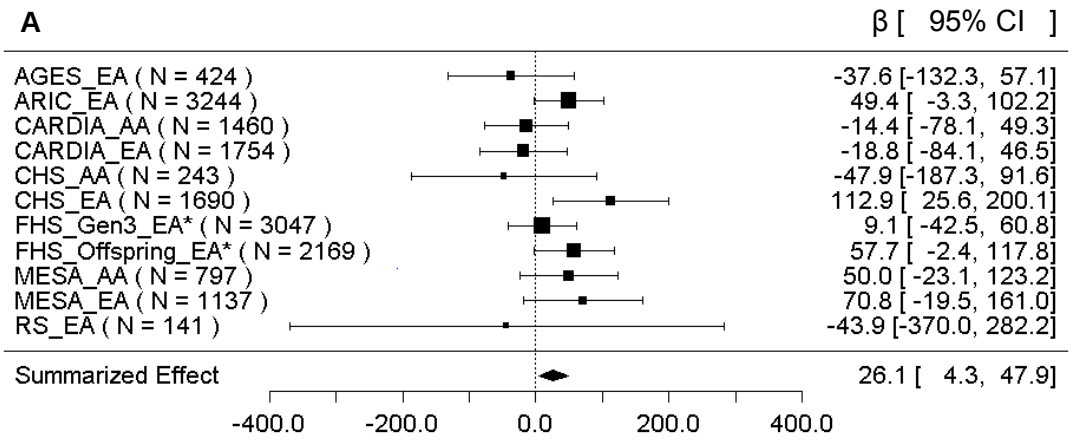
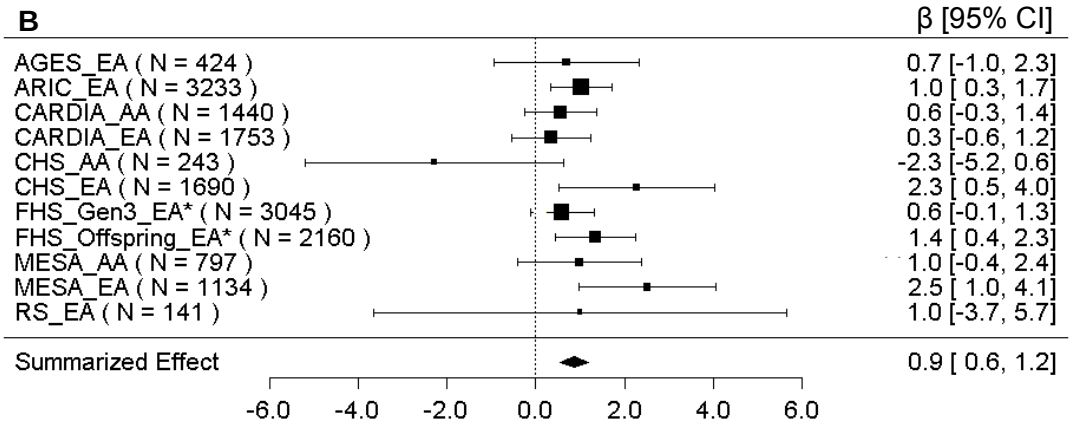


Figure E3. Forest plots of meta-analysis of the DHA biomarker on pulmonary function (sensitivity analysis of measurement time). Associations are presented for the DHA biomarker on (A) FVC and (B) FEV_1, only for the cohorts in which the spirometry test and omega-3 fatty acid biomarkers were measured within an average of one year. β (unit: mL) denotes the coefficient from the fixed-effects meta-analysis for DHA biomarker on the pulmonary function outcome per 1% (of total fatty acids) increment, with its 95% confidence interval. The vertical line in the center means no effect of the DHA biomarker on pulmonary function; β value to the right of the line means positive effect, while β value to the left of the line means negative effect. The size of black square for each cohort represents the variance of the β coefficient, so that cohorts with smaller variances have larger black squares. Cohorts listed in the forest plots are ordered based on alphabetical order, with sample size of each cohort shown in the parenthesis. *FHS has omega-3 fatty acid biomarkers measured in red blood cells, rather than plasma phospholipids.

Abbreviations: AA = African ancestry; AGES = Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC = Atherosclerosis Risk in Communities; CARDIA = Coronary Artery Risk Development in Young Adults; CHS = Cardiovascular Health Study; CI = Confidence interval; DHA = Docosahexaenoic acid; EA = European ancestry; FEV_1 = Forced expiratory volume in the first second; FVC = Forced vital capacity.



Interaction coefficient of DHA biomarker and current smoking on FEV_1

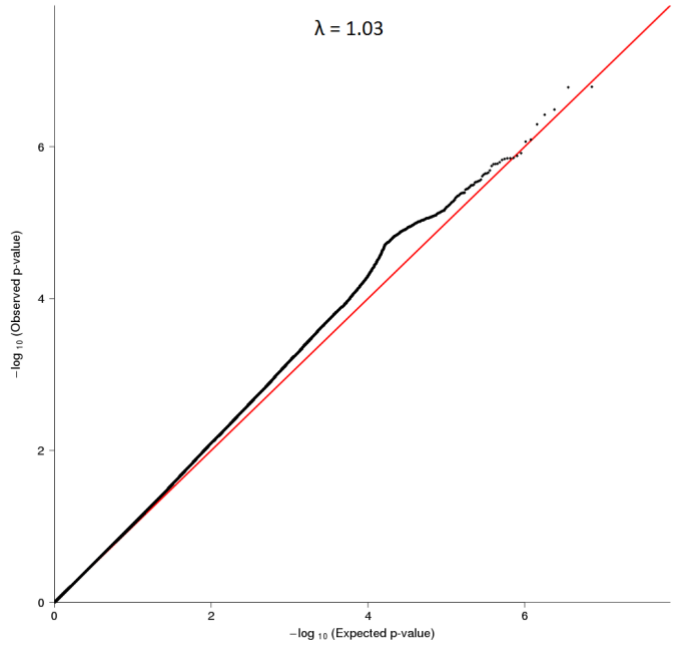


Interaction coefficient of DHA biomarker and current smoking on FEV_1/FVC

Figure E4. Forest plots of the interaction meta-analysis of DHA biomarker with current smoking status on pulmonary function. β denotes the interaction term coefficient of DHA biomarker with current smoking status on (A) FEV_1, and (B) FEV_1/FVC, compared to never smoking, from the fixed-effects meta-analysis, per 1% (of total fatty acids) increment of DHA biomarker, with its 95% confidence interval. The vertical line in the center means no effect of DHA on the pulmonary function outcome; β value to the right of the line means positive effect, while β value to the left of the line means negative effect. The size of black square for each cohort represents the variance of the β coefficient, so that cohorts with smaller variances have larger black squares. Cohorts listed in the forest plots are ordered based on alphabetical order, with sample size of each cohort shown in the parenthesis. *FHS has omega-3 fatty acid biomarkers measured in red blood cells, rather than plasma phospholipids.

Abbreviations: AA = African Ancestry; AGES = Age, Gene, Environment, Susceptibility Study—Reykjavik, Iceland; ARIC = Atherosclerosis Risk in Communities; CARDIA = Coronary Artery Risk Development in Young Adults; CHS = Cardiovascular Health Study; CI = Confidence interval; DHA = Docosahexaenoic acid; EA = European ancestry; FEV_1 = Forced expiratory volume in the first second; FHS (Offspring) = Framingham Heart Study—Offspring Cohort; FHS (Gen3) = Framingham Heart Study—Generation 3 Cohort; FVC = Forced vital capacity; MESA = Multi-Ethnic Study of Atherosclerosis; RS = Rotterdam (Netherlands) Study.

(A)



(B)

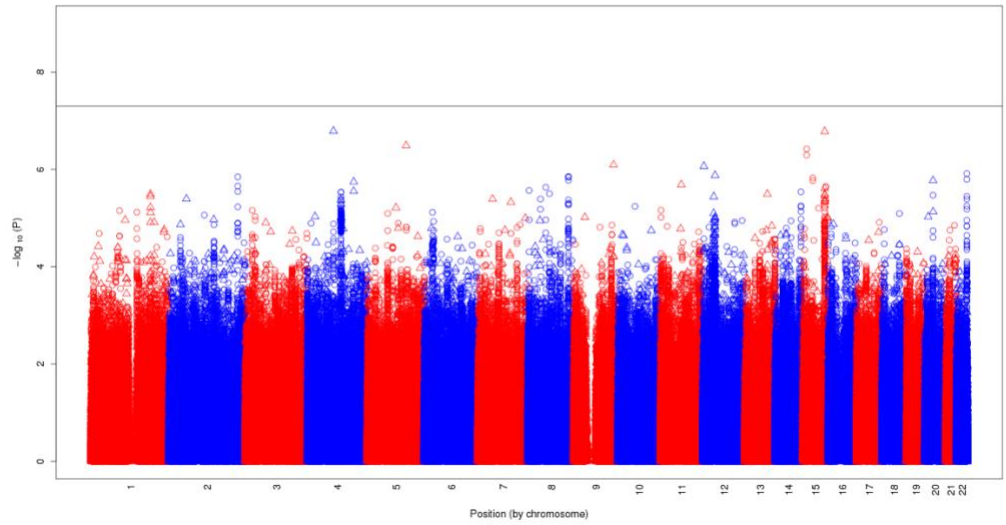
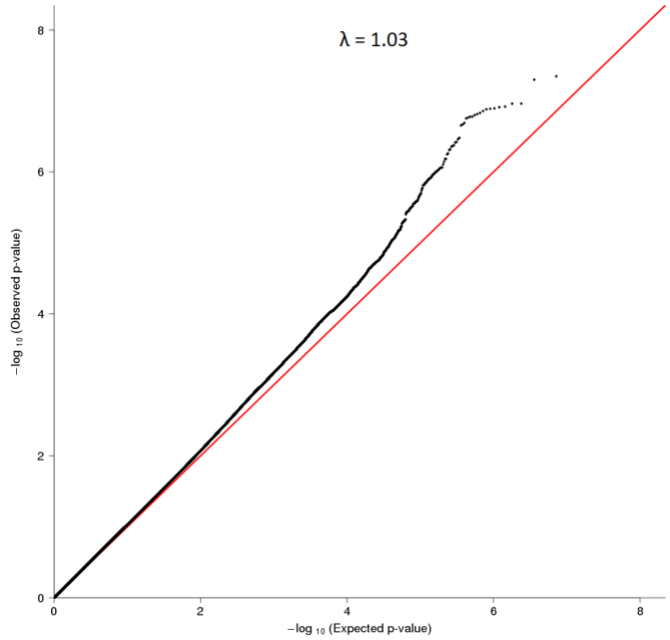


Figure E5. **Genome-wide SNP/indel associations with FEV_1, accounting for interaction with DHA, in cross-ancestry meta-analysis.** The -$\log_{10}$ (meta-analysis P_{2df}) for 7.2 million SNPs/indels with minor allele frequency > 5% and imputation quality > 0.3 are plotted against (A) expected P values where the red line depicts the null hypothesis of no association and (B) chromosomal position where the solid black line depicts the genome-wide statistical significance threshold ($P < 5\times10^{-8}$). SNPs/indels failing to achieve minimal frequency and imputation quality in all but one cohort were excluded from results.

(A)



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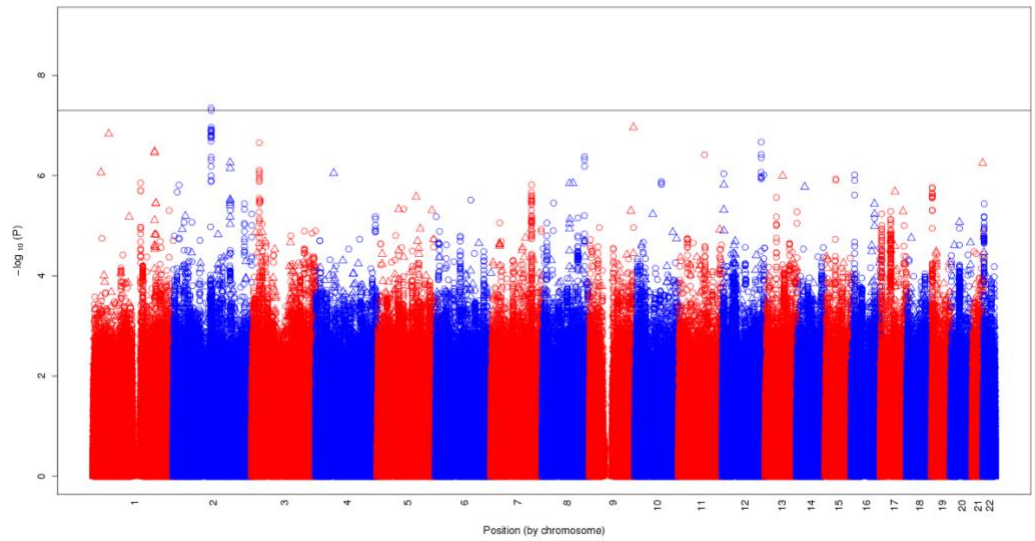
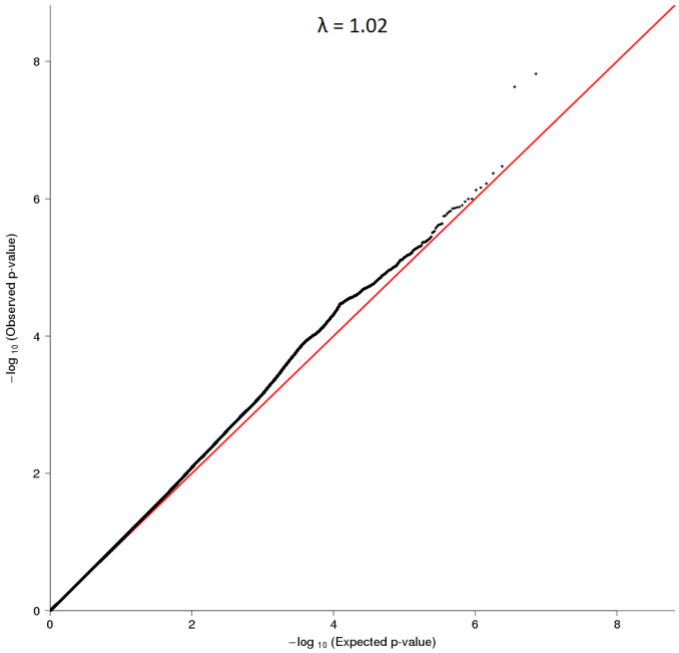


Figure E6. **Genome-wide SNP/indel associations with FVC, accounting for interaction with DHA, in cross-ancestry meta-analysis.** The -$\log_{10}$ (meta-analysis P_{2df}) for 7.2 million SNPs/indels with minor allele frequency > 5% and imputation quality > 0.3 are plotted against (A) expected P values where the red line depicts the null hypothesis of no association and (B) chromosomal position where the solid black line depicts the genome-wide statistical significance threshold ($P < 5\times10^{-8}$). SNPs/indels failing to achieve minimal frequency and imputation quality in all but one cohort were excluded from results.

(A)



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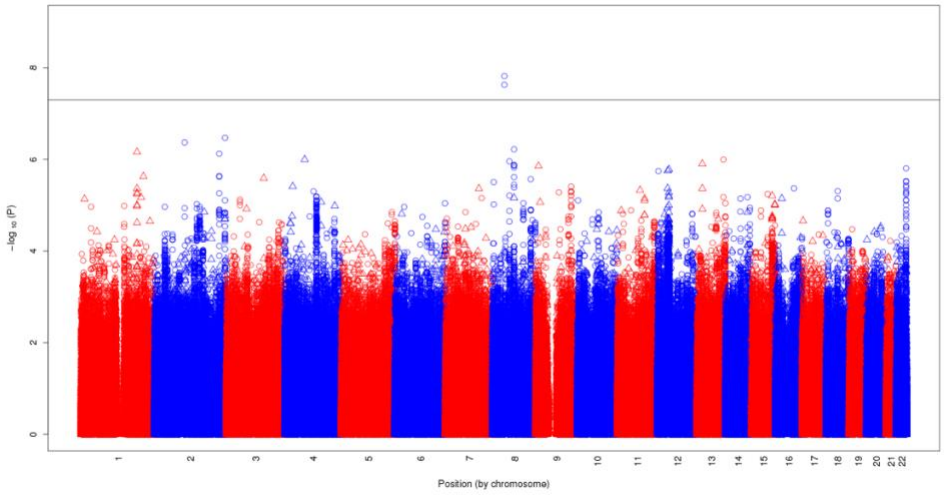
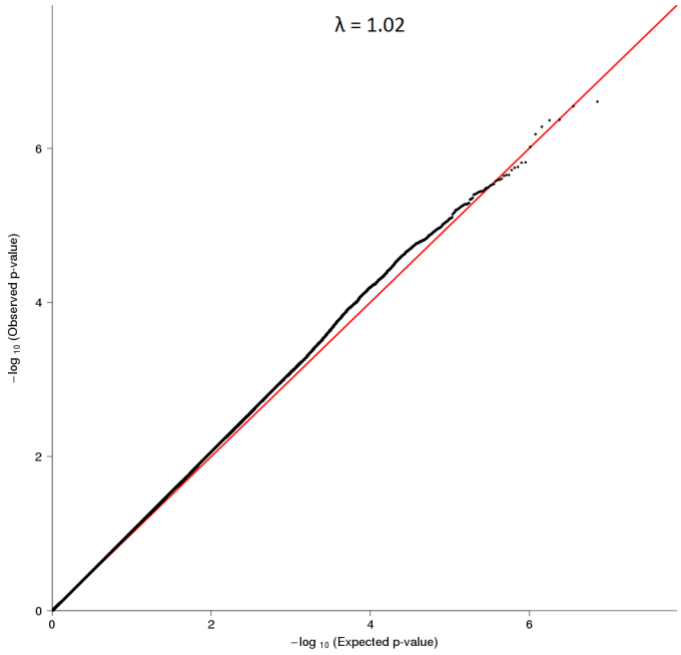


Figure E7. **Genome-wide SNP/indel associations with FEV_1, accounting for interaction with DPA, in cross-ancestry meta-analysis.** The -$\log_{10}$ (meta-analysis P_{2df}) for 7.2 million SNPs/indels with minor allele frequency > 5% and imputation quality > 0.3 are plotted against (A) expected P values where the red line depicts the null hypothesis of no association and (B) chromosomal position where the solid black line depicts the genome-wide statistical significance threshold ($P < 5\times10^{-8}$). SNPs/indels failing to achieve minimal frequency and imputation quality in all but one cohort were excluded from results.

(A)



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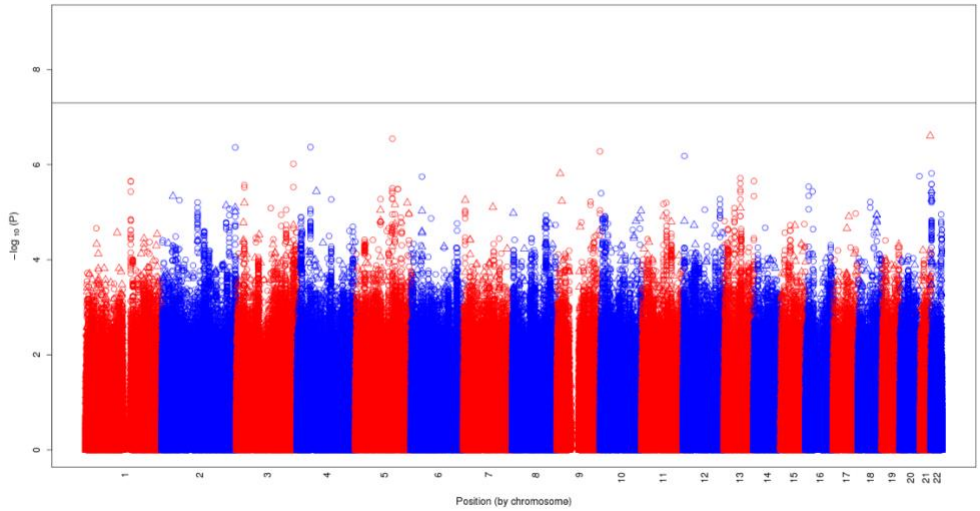
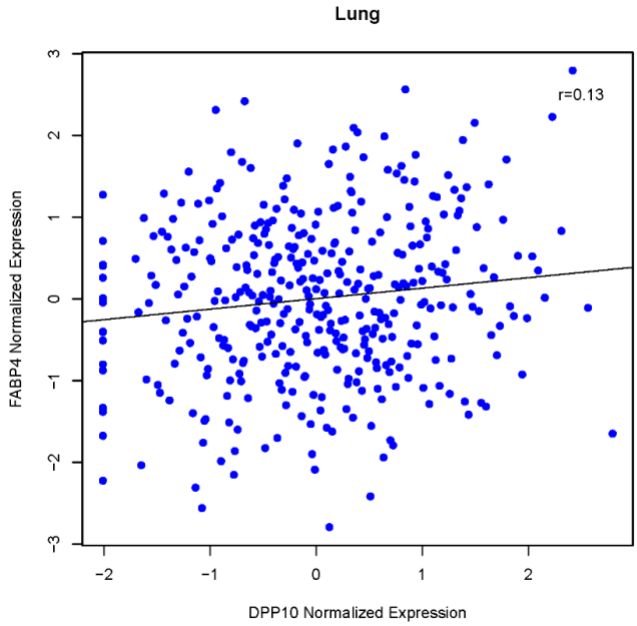


Figure E8. **Genome-wide SNP/indel associations with FVC, accounting for interaction with DPA, in cross-ancestry meta-analysis.** The -$\log_{10}$ (meta-analysis P_{2df}) for 7.2 million SNPs/indels with minor allele frequency > 5% and imputation quality > 0.3 are plotted against (A) expected P values where the red line depicts the null hypothesis of no association and (B) chromosomal position where the solid black line depicts the genome-wide statistical significance threshold ($P < 5\times10^{-8}$). SNPs/indels failing to achieve minimal frequency and imputation quality in all but one cohort were excluded from results.

(A)



(B)

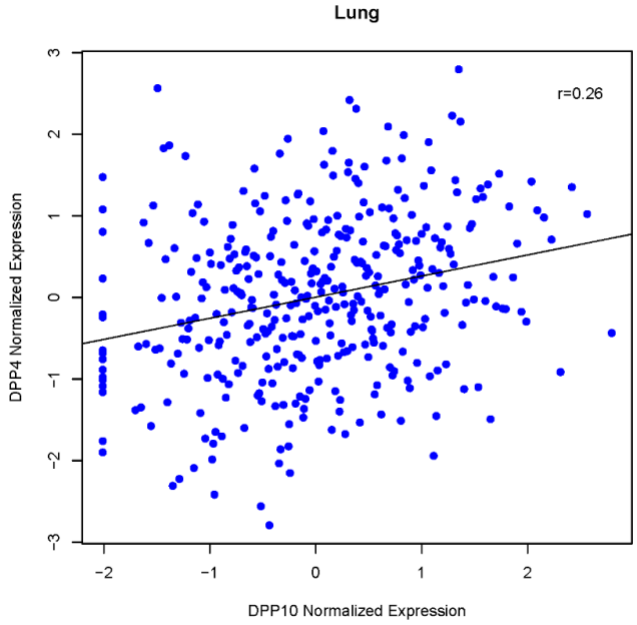


Figure E9. **Co-expressed genes with *DPP10* in lung tissue.** Scatterplots show the correlation between expression levels of *DPP10* and (A) *FABP4*, as well as (B) *DPP4*. Data are based on GTEx v7 RNA-Seq data (fully processed, filtered and normalized expression data, as previous described (28)). Pearson correlation coefficient, *r*, is shown.

Supplemental References

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CHAPTER 4

CHANGE IN PLASMA ALPHA-TOCOPHEROL ASSOCIATED WITH ATTENUATED PULMONARY FUNCTION DECLINE AND INFLUENCED BY *CYP4F2* MISSENSE VARIANT

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Abstract

Background: Vitamin E (vitE) is hypothesized to protect pulmonary function given its antioxidant activity but evidence is limited for the effects of vitE supplementation on pulmonary function decline and factor related to the variability in plasma tocopherol concentrations after vitE supplementation.

Objective: We investigated change in vitE concentration in plasma after vitE supplementation (hereafter, plasma response) for association with pulmonary function decline over time, and genetic and non-genetic factors related to variability in plasma response.

Design: We studied 1,144 participants in the Selenium and Vitamin E Cancer Prevention Trial (SELECT), a 4-arm randomized controlled trial of vitE and selenium (Se) supplementation in men to prevent prostate cancer. Plasma response was calculated using vitE concentrations measured at baseline and year 3 using gas chromatography-mass spectrometry. Pulmonary function (forced expiratory volume in the first second [FEV_1]) was measured via spirometry at annual study visits, and genotypes were assayed on a subset (n =555 men in the vitE-only arm) via the Illumina MEGA^{ex} array. An as-treated analysis tested the association of plasma response with annual rate of change in FEV_1 using mixed-effects linear regression; models were extended to test effect modification by smoking. Previously reported genetic variants and non-genetic factors were tested for association with plasma response.

Results: Plasma change in free-cholesterol-adjusted α-tocopherol was associated with attenuated decline in pulmonary function, and sensitivity analyses showed the attenuation was greater in participants with a positive plasma response and in adherent participants of the treatment. We found that a 1 SD *higher* plasma response (i.e., change of 4 μmol/mmol free-cholesterol-adjusted α-tocopherol) was associated with

~8.6 mL/year *slower* FEV_1 decline ($P = 0.027$); this effect size translates to about a quarter of the effect of one year of aging, but in the opposite direction. There was also evidence of modification by smoking such that the positive effect was mainly found in never and current smokers (9.6 mL/year and 11.3 mL/year slower FEV_1 decline per 1 SD *higher* plasma response), but not in former smokers. Non-genetic factors associated with a *greater* increase in α-tocopherol after supplementation included vitE and cholesterol measures at baseline (*lower* α-tocopherol, *higher* γ-tocopherol, *higher* free cholesterol), and European ancestry [EA] (compared to African ancestry [AA]). A previously reported missense variant in *CYP4F2*, rs2108622 (T minor allele frequency = 31% for EA and 13% for AA), was associated with 2.4 µmol/L *greater* increase in plasma α-tocopherol after supplementation ($P = 0.0032$).

Conclusions: This randomized trial shows that change in plasma vitE response to supplementation was associated with attenuated decline in pulmonary function in healthy males aged > 50 years, with effects mainly found in never and current smokers. For individuals with the rs2108622-*T* minor allele, the vitE effective dosage may be lower compared to individuals with the major allele [C].

Keywords: vitamin E, respiratory function tests, CYP4F2 protein, human, male, clinical trial, smoking, continental population groups, genome-wide association study

Introduction

In this study, we investigated the association of change in plasma biomarker in response to vitamin E (vitE) supplementation with pulmonary function decline. VitE is a lipid-soluble antioxidant nutrient that scavenges free radicals in cells which can cause damage to the lungs (1, 2). Among the 8 forms of vitamin E, alpha-tocopherol (α-TOH) is the most abundant form in blood circulation due to preferential post-absorption retention, while gamma-tocopherol (γ-TOH) is the major form in the U.S. diet (e.g., rich in vegetable oil and nuts). Animal studies reported that vitE downregulates inflammation by reversing the age-related defect in T cells and by inhibiting the production of prostaglandin E2 (3). Studies of dietary manipulation in mice confirmed the potential of vitE supplementation to combat oxidative stress (e.g., from smoking) and to improve pulmonary function (4, 5). Observational studies of vitE and pulmonary function in humans were primarily cross-sectional (i.e., measurements taken at a single time point), and findings were mixed. Several cross-sectional studies reported a positive association of vitE with pulmonary function (6-18), and others reported little to no association (9, 14, 19-26). Only two studies with longitudinal data on pulmonary function have been reported; one studied dietary intake of vitE and found a null association (7), while the other used a serum vitE biomarker and reported an association of lower baseline vitE with faster pulmonary function decline, when focusing on heavy smokers (27). To our knowledge, only one prior RCT, using the Respiratory Ancillary Study (RAS) to the Selenium and Vitamin E Cancer Prevention Trial (SELECT) with 1,641 men, investigated the effect of vitE supplementation on longitudinal pulmonary function decline using an intent-to-treat analysis; the direction of effect was consistent with supplementation attenuating pulmonary function decline (-33 mL/year pulmonary function decline in the vitE arm *versus* -39 mL/year in the placebo arm), but the effect was not statistically significant

(P = 0.19) with no evidence for an interaction of vitE supplementation with smoking status (28).

Randomized controlled trials (RCTs), when feasible, provide high-quality evidence to infer causal effects of nutrition on chronic disease outcomes. There are several high-profile examples of nutrient—disease associations that were hypothesized based on mechanistic evidence from animal studies, confirmed in observational studies, but then showed null or even harmful effects when studied in an RCT design using intent-to-treat analysis (29-31). However, even though an RCT intent-to-treat design preserves balanced participant characteristics across study arms by analyzing the participants based on their initial treatment assignment (32), it may have some limitations depending on factors such as intervention timing relative to disease natural history, intervention dosage (33), non-compliance, and selected population characteristic including baseline nutriture and individual genetic variation. Thus, the efficacy of nutrient supplementation might vary across subgroups of the population and a precision nutrition or a personalized nutrition approach may be informative, analogous to the concept of precision medicine (34).

As described above, a null result in an intent-to-treat analysis of an RCT of nutrient supplementation may be a weak test of the hypothesis due to failure to account for baseline nutrient status, non-compliance and/or inter-individual variation in response to supplement given genetic differences. Indeed, for vitE supplementation, an *in silico* simulation study reported that many genes related to vitE metabolism are highly polymorphic (35) making it plausible that variants in these genes may contribute to inter-individual variation in response to supplementation. Furthermore, a few genome-wide association studies (GWAS) reported several genetic variants associated with cross-sectional plasma vitE level (36-38), and one study (a male heavy-smoker population) reported two genome-wide significant genetic variants

associated with plasma α-TOH concentration after 3 years of vitE supplementation (39).

In this study, we conducted an 'as-treated' analysis of vitE supplementation effects on pulmonary function decline in RAS to test whether a greater response to vitE supplementation (i.e., greater change in plasma vitE level) was associated with attenuation in pulmonary function decline in a generally healthy male population. Next, we investigated genetic and non-genetic factors of inter-individual variation in plasma response to vitE supplementation (i.e., change in plasma vitE) to identify sub-populations that may benefit differentially from vitE supplementation to improve their lung health.

Methods

Study Design and Participants

RAS is an ancillary study to SELECT, a phase 3 randomized controlled double-blinded trial of prostate cancer prevention in 35,533 men in the United States, Canada, and Puerto Rico aged ≥ 50 years for African Americans or > 55 years for all other men. At the study baseline for SELECT, participants had no prostate cancer diagnosis or suspicion of cancer, a serum prostate-specific antigen level ≤ 4 ng/mL, no more than 175 mg/day acetylsalicylic acid or 81 mg/day of acetylsalicylic acid with clopidogrel bisulfate taken for anticoagulant therapy, a normal blood pressure, and no history of hemorrhagic stroke (40).

RAS included men from 16 SELECT sites, and the randomized design was preserved in RAS. The four study arms were (1) vitE [400 IU/day of *all rac*-α-tocopheryl acetate] + selenium (Se) [200 μg/day of L-selenomethionine] (vitE+Se arm); (2) vitE + Se placebo (vitE arm); (3) vitE placebo + Se (Se arm); and (4) vitE placebo + Se placebo (double placebo arm). The Cornell University IRB and each of

the 16 study sites' IRB approved the study. RAS enrolled 2,921 participants, and 2,846 participants had ≥ 1 pulmonary function measurement that met American Thoracic Society (ATS) standardization of spirometry guidelines. Among these men, the current study focused on 1,144 participants of European or African ancestry in the two vitamin E arms (vitE+Se, vitE), using plasma vitE that was assayed on blood samples from the RCT baseline and after 3 years (**Supplemental Figure 1**). Participants of other ancestries (e.g., Hispanics) or races (e.g., Pacific Islanders, Asian) were excluded (total $n = 86$) given their small sample sizes.

Pulmonary Function

Pulmonary function, including forced expiratory volume in the first second (FEV_1), was measured via the EasyOne handheld spirometer, which was previously tested for validity and reliability by comparing it against a reference laboratory-based spirometer (41). The primary endpoint in RAS was annual rate of change in FEV_1. Pulmonary function tests (PFTs) were performed at annual SELECT visits in the RAS participants. Since RAS was a post-randomization ancillary study to SELECT, there were no PFTs prior to randomization. In addition, because SELECT was discontinued in October 2008 due to no benefit of the intervention on prostate cancer, only PFTs measured prior to March 1, 2009 were included to provide a conservative estimate of possible effects of intervention (4 month beyond the termination of supplements). Moreover, due to the discontinuation of SELECT, 65% of RAS participants completed ≥ 2 PFTs, leading to a loss of power in the intent-to-treat analysis (28). Given that trial termination was an independent event unrelated to the timing of the annual study visits of the participants, we assume that the RAS men with ≥ 2 PFTs is a random sample of the entire RAS population. Only PFTs meeting ATS standardization criteria were included, and repeated PFTs had to be at least 2 years apart to be included.

Vitamin E

Plasma vitE was assayed in samples from men in vitE arms of the study (VitE arm, VitE+Se arm) and in a random sample of men in the double placebo arm (*n* = 92). Blood samples were collected at baseline and at the year 3 annual visit and stored at -80°C for a maximum of 9 years (**Supplemental Methods**). Plasma α-TOH, γ-TOH and free un-esterified cholesterol were assayed via gas chromatography-mass spectrometry (GC-MS) on a Hewlett Packard 6890 gas chromatograph coupled with a Hewlett Packard 6890 mass spectrometer (Palo Alto, CA). Free cholesterol was measured in this study, as a proxy for total cholesterol given that the ratio of free to total cholesterol is relatively constant (42). Hereafter, any free cholesterol-adjusted tocopherols refers to the adjustment of free un-esterified cholesterol. Both raw tocopherol and free-cholesterol-adjusted tocopherol were used in this study, because as a lipid-soluble nutrient, plasma vitE concentration often varies by blood lipid levels (43).

Genotyping

Genome-wide genotypes were assayed on a subset (*n* = 625 unrelated men in the vitE arm), and we used these data to identify genetic variants that affected plasma response to vitE supplementation. The vitE arm was chosen to isolate these effects from potential synergistic effects of Se supplementation. Blood buffy coats for participants in the vitE arm were stored in a freezer at -80°C in the Human Metabolic Research Unit, Cornell University. In 2011, DNA was extracted from peripheral blood leukocytes via the Puregene Blood Kit chemistry on an Autopure LS automated DNA purification instrument (Qiagen, Valencia, CA) by the Center for Inherited Disease Research Laboratory. The quantification and normalization of DNA samples was completed by the Biotechnology Resource Center, Cornell University. DNA concentration were quantitated using the Quantifluor dsDNA kit via the Spectra Max

M2 plate reader (Molecular Devices, San Jose, CA). Samples were re-suspended in the hydration buffer (10mM Tris-Cl, 1mM EDTA, pH 8.0) and sent to the Northwest Genomics Center at University of Washington on dry ice in April 2016 for genotyping.

The Illumina Infinium® Expanded Multi-Ethnic Genotyping Array (MEGA^{ex}) (Illumina Inc., San Diego, CA, USA) was used to genotype more than 2 million genetic markers in 618 participants (7 participants were excluded due to very low DNA concentration). After excluding participants with failed call rate (n =15), genotyped data were available for 603 participants (440 European ancestry participants [EAs] and 141 African ancestry participants [AAs]). Quality control (QC) was applied at both the individual level and the single nucleotide polymorphism (SNP) level. At the individual level, the QC filters included (1) missing call rate > 3%, (2) duplicate sample (identity-by-state > 0.9), (3) first degree relative (identity-by-descent > 0.4), and (4) excessive homozygosity, which led to exclusion of 9 EAs and 1 AA, leaving 431 EAs and 140 AAs. For the genetic analysis of plasma change in tocopherols, the final sample size with all covariate information available was 555 (N_{EA} = 417, and N_{AA} = 138). At the SNP level, the QC filters included (1) missing rate > 3%, (2) Hardy-Weinberg equilibrium p-value < 1×10^{-4} and (3) duplicate SNPs, which reduced the total SNP count from 2,036,060 to 1,671,215 for EAs and 1,662,290 for AAs.

Genotype imputation was performed based on the Haplotype Reference Consortium panel (version r1.1 2016) (44) using the Michigan Imputation Server. Given the sample size, we focused on variants with minor allele frequency (MAF) > 0.03 in both the ancestry-relevant 1000 Genomes Phase 3 super population (EUR for EAs and AFR for AAs) and the study population, resulting in a final set of 6,069,665 variants for EAs and 8,944,804 variants for AAs.

Statistical Analysis of Plasma Vitamin E—Pulmonary Function Association

An as-treated analysis investigated the association of change in plasma tocopherol concentration (α-TOH and γ-TOH) in response to vitE supplementation with annual rate of change in FEV_1 in the two vitE arms. A mixed-effects linear regression model was used to account for the repeated PFTs for each participant. The FEV_1 value of each participant at each time point was the dependent variable in the model, with a variable for time as an independent variable that reflected the effect of one year of aging on change in FEV_1 (i.e., annual rate of change in FEV_1). The association of change in plasma vitE concentration with annual rate of change in FEV_1 was modeled as an interaction term between change in plasma vitE and the time variable in the linear mixed-effects model (see equation below). The association was investigated both with and without adjustment for other covariates, which included PFT-related factors such as age, height, race, smoking status, smoking dose (current smokers only), as well as vitE status-related factors such as treatment arm assignment, and baseline α-TOH, γ-TOH, and free cholesterol concentrations.

$$FEV_{1\,ij} = \beta_0 \text{ (intercept)} + \beta_1 \times \text{time}_{ij} + \beta_2 \times \Delta \text{ plasma vitE}$$
$$+ \beta_3 \times \Delta \text{ plasma vitE} \times \text{time}_{ij} + \mu_i \text{ (random effect of subject/individual)}$$
$$+ \varepsilon_{ij} \text{ (random error)} + \text{(other covariates)}$$
$$\text{where } i = i^{\text{th}} \text{ individual, } j = j^{\text{th}} \text{ PFT measure}$$

Sensitivity analyses were explored to sharpen the estimation of the association of vitE with FEV_1. First, the analysis was limited to participants who had an increase in plasma α-TOH after vitE supplementation (i.e., positive responders to supplementation; ~25% had a decrease, which could potentially be due to non-adherence or an intake of higher-dose vitE supplementation before the participant enrollment in the trial). Then, the analysis was further limited to men who were adherent to the intervention, defined by self-reported use of ≥ 80% of the study

supplement pills. The significance threshold for all analyses was set at two-sided $P \leq 0.05$. Furthermore, non-linearity of the association was examined by including a square term for the change in alpha-tocopherol variable (hereafter referred to as Δα-TOH) and the influence of one outlier for Δα-TOH was examined by comparing the results with and without the outlier.

Possible effect modification of the change in plasma vitE—FEV_1 association by smoking status, by treatment arm, and by race was examined in separate models via a 3-way interaction term (each putative effect modifier × Δ plasma vitE × time). Stratified analysis of each effect modifier was explored if the interaction p-value was ≤ 0.05.

Statistical Analysis of Non-Genetic Factors—Plasma Response to Supplementation

We did a systematic search of the literature for reports of non-genetic factors associated with plasma vitE concentration. For each factor identified, a further literature review sought to identify the biological plausibility of the putative association (**Supplemental methods**), leading to a final set of factors that were tested individually and then simultaneously in joint models. The variables tested were race, treatment arm assignment, and baseline values for age, body mass index (BMI), α-TOH concentration, γ-TOH concentration, free cholesterol concentration, plasma Se concentration, and dietary intakes of vitamin C, fiber, alcohol, and smoking status measured over the course of the study. Linear regression models in SAS (version 9.4, SAS Institute, Cary, NC) tested the association of both raw Δα-TOH and free-cholesterol-adjusted Δα-TOH. The total R^2 denoted the percent of variability in change in vitE concentration explained by the full model, and the individual R^2 denoted the estimated variability in the outcome explained by each individual factor (e.g., race, baseline tocopherol concentration), which was calculated as the difference between total R^2 with and without the factor in the model.

Statistical Analysis of Genetic Variant—Plasma Response to Supplementation

Agnostic (i.e., GWAS) and hypothesis-based (i.e., candidate gene) analyses were conducted in this study to identify genetic factors that are associated with plasma response to vitE supplementation.

GWAS analyses were stratified by race (n = 417 for EAs and n = 138 for AAs) and then meta-analyzed for each of four vitE phenotypes (Δα-TOH, free-cholesterol-adjusted Δα-TOH, Δγ-TOH, and free-cholesterol-adjusted Δγ-TOH) (45) in models adjusted for age, baseline tocopherol concentration, and principal components to account for population genetic substructure. We investigated associations of two SNPs, which were identified at a genome-wide significance level defined in a previous GWAS of post-vitE-supplementation plasma α-TOH among heavy smokers ($P < 5\times10^{-7}$, accounting for 549,989 tested SNPs) (39), and applied a Bonferroni-corrected p-value threshold of 0.025 (α=0.05/2 SNPs). Replicated SNPs were further examined in models that included additional adjustment for the set of previously reported non-genetic covariates (**Supplemental Methods**), which were not included in our parsimonious GWAS model that was designed to optimize statistical power.

The candidate gene analysis identified 42 genes related to vitE metabolism via literature search (**Supplemental Table 1**). The gene-based P-value was computed from the P-values of all SNPs that were annotated based on physical proximity to genes (+/- 20 kilobases of the transcription start and stop sites) via MAGMA (46) using the FUMA tool (47). The Bonferroni-corrected P-value threshold for the gene-based analysis was set at 0.0012 for testing 42 candidate genes (α=0.05/42).

Results

Participant Characteristics

Among all the participants enrolled in RAS (*n* = 2,921, **Supplemental Figure 1**), only 74 withdrew (~2.5%). For the model of annual rate of change in FEV_1, 1,144 participants were included: N_{EA} = 874 (76.4%) and N_{AA} = 270 (23.6%). Average age was 63 years, and a history of cigarette smoking was common (49% former smokers and 19% current smokers, **Table 1**). The majority of participants were overweight and/or obese: 48% had BMI of 25-29.9 and 34% had BMI ≥30.

The mean (SD) *increase* in plasma α-TOH after 3 years of vitamin E supplementation was 8.2 (12.5) and 7.4 (14.5) μmol/L in the vitE arm and vitE+Se arm, respectively, whereas the average in the double placebo was significantly lower than zero [mean change: -5.3 μmol/L α-TOH (SD = 16.8), *P* = 0.0032] (**Table 2**). γ-TOH decreased with supplementation by an average of 1.7 μmol/L in both vitE arms (SD = 2.4 in the vitE arm and 2.6 in the vitE+Se arm), with little or no change in the double placebo arm [mean change: +0.5 μmol/L (SD = 2.9)] (**Table 2**). Six percent of participants were at risk of vitE deficiency at baseline using the clinical threshold of 12 μmol/L (48), which dropped to 2% after 3-year supplementation in the vitE arms. Participants in the vitE arms did not differ in their baseline characteristics or in change in plasma tocopherol concentrations (**Tables 1 and 2**). While there was little to no correlation between baseline α-TOH and γ-TOH in participants in the two vitE arms (Pearson *r* = 0.018), there was an inverse correlation between Δα-TOH and Δγ-TOH (Pearson *r* = -0.22).

Plasma Δα-TOH and Longitudinal Pulmonary Function Decline

Plasma Δα-TOH was associated with longitudinal FEV_1 decline (**Table 3**), but there was little to no association of Δγ-TOH (**Supplemental Table 2**). Specifically, in the fully adjusted model, 1 μmol/mmol increase in free-cholesterol-adjusted Δα-TOH was

Table 1. Characteristics of male participants with vitamin E supplementation at baseline and year 3 in the Respiratory Ancillary Study (*n* = 1,144) [1]

	Overall (*n* = 1144)	**Vitamin E + Selenium (*n* = 565)**	**Vitamin E + Placebo (*n* = 579)**	**$P_{vitE+Se\ vs\ vitE}$ [2]**
Age (y)	62.6 (6.4)	62.7 (6.3)	62.5 (6.4)	0.57
Ethnicity, N (%) [3]				0.64
European American	874 (76)	435 (77)	439 (76)	
African American	270 (24)	130 (23)	140 (24)	
Smoking Status, N (%) [3,4]				0.98
Never	364 (32)	181 (32)	183 (32)	
Former	564 (49)	277 (49)	287 (50)	
Current	216 (19)	107 (19)	109 (19)	
BMI (kg/m^2)	28.9 (4.6)	28.4 (4.6)	29.3 (4.6)	**0.0019**
Height (cm)	176.6 (7.1)	176.6 (7.0)	176.7 (7.1)	0.83
FEV_1 (mL)	2986.8 (680.8)	2990.4 (669.1)	2983.2 (692.5)	0.86

[1] Data are presented as mean (standard deviation [SD]) in the table, and measured at baseline, unless otherwise noted.
[2] P-value is based on the t test for continuous variables or based on the chi-square test for categorical variables in the vitamin E arms (vitE+Se arm versus vitE arm). Significant $P < 0.05$ is in bold.
[3] Data are presented as number (percentage) for ethnicity and smoking status.
[4] Here it refers to the smoking status throughout the study till last pulmonary function measurement of each participant.
Abbreviations: BMI, body mass index; FEV_1, forced expiratory volume in the 1^{st} second, vitE, vitamin E supplementation only arm; vitE+Se, vitamin E + selenium supplementation arm.

Table 2. Plasma tocopherol concentrations in male participants in the two vitamin E arms as well as in the placebo arm of the Respiratory Ancillary Study [1]

	Baseline				Change after 3-year supplementation [2]			
	Double Placebo (*n* = 92)	Vitamin E + Selenium (*n* = 565)	Vitamin E + Placebo (*n* = 579)	$P_{vitE+Se\ vs\ vitE}$ [3]	Double Placebo (*n* = 92)	Vitamin E + Selenium (*n* = 565)	Vitamin E + Placebo (*n* = 579)	$P_{vitE+Se\ vs\ vitE}$ [3]
α-tocopherol								
- Unadjusted (μmol/L)	25.1 (22.3)	22.3 (9.8)	21.5 (9.0)	0.16	-5.3 (16.8)	7.4 (14.5)	8.2 (12.5)	0.28
- Free-cholesterol-adjusted (μmol/mmol chol)	10.2 (4.5)	9.3 (3.5)	9.1 (3.1)	0.38	-1.3 (3.4)	3.6 (4.7)	3.5 (4.1)	0.77
γ-tocopherol								
- Unadjusted (μmol/L)	3.3 (2.9)	3.4 (2.6)	3.5 (2.4)	0.43	0.5 (2.9)	-1.7 (2.6)	-1.7 (2.4)	0.63
- Free-cholesterol-adjusted (μmol/mmol chol)	1.4 (0.9)	1.4 (0.9)	1.5 (0.9)	0.054	0.2 (0.9)	-0.7 (0.9)	-0.7 (0.9)	0.60
Vitamin E deficiency, No. (%) [4]	6 (6.5%)	31 (5.5%)	37 (6.4%)	0.52				

[1] Data are presented as mean (standard deviation [SD]) in the table, unless otherwise noted. Vitamin E supplementation refers to 400 IU/day *all rac-α*-tocopherol, and includes participants randomized to vitamin E + selenium and to vitamin E + placebo.
[2] Change in tocopherol concentration was calculated as tocopherol concentration at baseline (or pre-supplementation) subtracted from that at year 3. For change in free-cholesterol-adjusted tocopherol concentration, it was calculated as the difference between the tocopherol concentration at year 3 divided by free cholesterol concentration at year 3 and the tocopherol concentration at baseline divided by free cholesterol concentration at baseline.
[3] P-value is based on the t test for continuous variables or based on the chi-square test for categorical variables in the vitamin E arms (vitE+Se arm versus vitE arm).
[4] At risk of vitamin E deficiency defined as <12 μmol/L α-tocopherol (48) at study baseline.
Abbreviations: μmol/mmol chol, μmol/mmol cholesterol; vitE, vitamin E supplementation only arm; vitE+Se, vitamin E + selenium supplementation arm.

Table 3. Association of plasma change in α-tocopherol after 3-year vitamin E supplementation with annual rate of change in FEV_1 (*n* = 1,144)

	Full sample [1] (*n* = 1,144)			**Sample with increased α-TOH [1,2] (*n* = 857)**			**Adherent sample with increased α-TOH [1,2,3] (*n* = 609)**		
	Unadjusted β (SE) [4]	Adjusted β (SE) [4,5]	P [6]	Unadjusted β (SE) [4]	Adjusted β (SE) [4,5]	P [6]	Unadjusted β (SE) [4]	Adjusted β (SE) [4,5]	P [6]
ΔRaw α-TOH	0.23 (0.20)	0.23 (0.20)	0.26	0.46 (0.29)	0.46 (0.29)	0.13	0.59 (0.34)	0.59 (0.35)	0.088
ΔAdjusted α-TOH [7]	0.91 (0.60)	0.96 (0.60)	0.11	1.01 (0.94)	1.32 (0.79)	0.097	2.07 (0.96)	2.16 (0.97)	**0.027**

[1] All participants in the vitamin E + selenium arm and in the vitamin E + placebo arm.
[2] Positive change in plasma α-tocopherol concentration after 3 years of *all rac-α*-tocopherol supplementation.
[3] Adherent participants (defined as self-report of taking at least 80% of the supplement pills) during the 3 years of supplementation.
[4] β was the coefficient of the Δα-TOH × time term in the statistical model.
[5] Adjusted for age at baseline, height, race, smoking status, smoking dose at baseline, treatment arm, baseline α-tocopherol, baseline γ-tocopherol, and baseline free cholesterol (only in the model with raw α-tocopherol values).
[6] P-value of the Δα-TOH × time term in the model adjusted for other covariates. Significant $P < 0.05$ is in bold.
[7] ΔAdjusted α-TOH refers to the change in free-cholesterol-adjusted α-tocopherol concentration from baseline to year 3.

associated with a 0.96 mL/year (SD = 0.60) attenuation in annual FEV_1 decline in the full sample with P-value of 0.11. Sensitivity analyses sought to further define the association by limiting the models as follows: including only men who responded to supplementation with an increase in Δα-TOH [+1.32 mL/year (SE = 0.79), $P = 0.097$]; and including only positive responders with self-reported adherence to the intervention [+2.16 mL/year (SD = 0.97), $P = 0.027$]. Overall, there was strong evidence that an increase in free-cholesterol-adjusted Δα-TOH was associated with an attenuation in annual FEV_1 decline, and there was no evidence of non-linearity in any of the models above. In positive responders who were adherent to the intervention, a 1 SD increase in plasma Δα-TOH (~4 μmol/mmol free cholesterol) was associated with an attenuation of ~8.6 mL/year in FEV_1 decline. To put the magnitude into context, annual rate of decline in FEV_1 was 35 mL/yr and thus an increase of 1 SD in plasma free-cholesterol-adjusted α-TOH is about one-quarter of the effect of one year of aging on FEV_1, but in the opposite direction. Consistent trends were observed for Δα-TOH (without adjusting free cholesterol; **Table 3**). There was one outlier in the plasma alpha-tocopherol data, but the removal of this outlier did not change the findings.

Smoking status modified the association of plasma Δα-TOH with FEV_1 decline in men receiving vitE supplementation ($P_{\text{smoking interaction}} < 0.05$, **Supplemental Table 3)**. Stratified analysis confirmed that an increase in plasma free-cholesterol-adjusted Δα-TOH was significantly associated with attenuation in FEV_1 decline in never smokers ($P = 0.019$) and borderline significant in current smokers ($P = 0.078$), but not in former smokers ($P = 0.45$, **Table 4**). To put the effect magnitude in context, 1 SD higher plasma free-cholesterol-adjusted Δα-TOH (~4 μmol/mmol free cholesterol) was associated with 9.6 mL/yr and 11.3 mL/yr attenuation in FEV_1 decline in never and current smokers, respectively. The same trend was observed for unadjusted Δα-TOH

Table 4. Association of plasma change in α-tocopherol after 3-year vitamin E supplementation with annual rate of change in FEV_1, stratified by smoking status (*n* = 1,144) [1]

	ΔRaw α-TOH		ΔAdjusted α-TOH [2]	
	β (SE) [3]	P [4]	β (SE) [3]	P [4]
Smoking status				
- Never smokers (***n*** = 364)	0.83 (0.35)	**0.018**	2.41 (1.02)	**0.019**
- Former smokers (***n*** = 564)	-0.41 (0.29)	0.16	-0.64 (0.84)	0.45
- Current smokers (***n*** = 216)	0.76 (0.50)	0.13	2.83 (1.60)	*0.078*

[1] All participants in the vitamin E + selenium arm and in the vitamin E + placebo arm. The model has been adjusted for age at baseline, height, race, smoking status, smoking dose at baseline, treatment arm, baseline α-tocopherol, baseline γ-tocopherol, and baseline free cholesterol (only in the model with raw α-tocopherol).
[2] ΔAdjusted α-TOH refers to the change in free-cholesterol-adjusted α-tocopherol concentration from baseline to year 3.
[3] β was the coefficient of the Δα-TOH × time term in the statistical model for each smoking stratum.
[4] P-value of the Δα-TOH × time term in the model adjusted for other covariates. Significant $P < 0.05$ is in bold and $P < 0.10$ is italicized.

(**Table 4**). Sensitivity analysis limited to men adherent to the intervention yielded similar findings (**Supplemental Table 4**). Neither race (AA, EA) nor treatment arm (vitE, vitE+Se) modified the Δα-TOH—FEV_1 decline association ($P_{interaction}$ = 0.85 and 0.43 for race and treatment arm, respectively).

Non-Genetic Factors and Plasma Δα-TOH

Plasma Δα-TOH values ranged from -55 µmol/L to +109 µmol/L in the two vitE arms [mean(SD) of 7.8 (13.5), with no difference between arms, $P = 0.68$]. *Higher* α-TOH at the study baseline was associated with a *lower* increase in plasma α-TOH ($P < 0.0001$) in response to supplementation, while *higher* free cholesterol and *higher* γ-TOH at baseline were associated with a *greater* increase in plasma α-TOH ($P < 0.0001$) (**Table 5**). Race was associated with plasma Δα-TOH ($P < 0.0001$): compared to EA men, AA men had a lower increase in plasma α-TOH concentration in response to supplementation. There was little to no association between age, BMI, and smoking status with plasma Δα-TOH ($P > 0.15$). Other dietary factors, including baseline plasma Se concentration, dietary intake of vitamin C, fiber, and alcohol, had little to no association with plasma Δα-TOH ($P > 0.2$). Consistent findings were observed when the outcome was the plasma free-cholesterol-adjusted Δα-TOH (**Table 5**). The fully adjusted model explained 18.7% of the variability (R^2) in plasma Δα-TOH in the two vitamin E arms (n = 1,144), in which baseline concentrations of α-TOH, γ-TOH, free cholesterol and race accounted for 10.9%, 1.7%, 1.2%, and 1.6% of the variability, respectively (**Table 5)**.

Genetic Factors and Plasma Δα-TOH

The mean increase in plasma α-TOH after 3-year supplementation was significantly higher in EAs versus AAs in the vitE arm ($P_{EA\ vs\ AA}$ = 0.010, 9.0 µmol/L *versus* 5.9 µmol/L), with similar direction, but lower magnitude differences in the vitE+Se arm ($P_{EA\ vs\ AA}$ = 0.60, 7.5 µmol/L *versus* 6.9 µmol/L). Genome-wide

Table 5. Factors associated with plasma change in α-tocopherol after 3-year vitamin E supplementation in the Respiratory Ancillary Study (*n* = 1,144) [1]

	ΔRaw α-TOH [2]		ΔAdjusted α-TOH [3]	
	β (SE) [4]	P-value [5]	β (SE) [4]	P-value [5]
Baseline α-tocopherol [6]	-0.64 (0.052)	**<0.0001**	-0.38 (0.039)	**<0.0001**
Baseline γ-tocopherol [6]	0.82 (0.17)	**<0.0001**	1.31 (0.14)	**<0.0001**
Cholesterol, µmol/L	0.0024 (0.00058)	**<0.0001**		
Race		**Type III < 0.0001**		**Type III < 0.0001**
- **White**	4.43 (0.93)	**<0.0001**	1.71 (0.30)	**<0.0001**
- **African American**	ref	ref	ref	ref
Treatment arm		Type III = 0.68		Type III = 0.21
- **Vitamin E + Selenium**	-0.30 (0.73)	0.68	0.30 (0.24)	0.21
- **Vitamin E + Placebo**	ref	ref	ref	ref
Age, year	-0.078 (0.059)	0.19	0.0082 (0.019)	0.67
Body Mass Index (BMI), kg/m^2	0.044 (0.080)	0.58	0.013 (0.026)	0.63
Smoking Status		Type III = 0.53		Type III=0.40
- **Current Smokers**	-1.16 (1.09)	0.29	-0.46 (0.35)	0.21
- **Former Smokers**	-0.11 (0.83)	0.89	-0.039 (0.27)	0.88
- **Never Smokers**	ref	ref	ref	ref
Baseline selenium concentration, parts per billion	-0.0024(0.0056)	0.67	-0.00064 (0.0018)	0.72
Total vitamin C intake, mg	-0.00041 (0.0014)	0.78	0.00025 (0.00046)	0.60
Dietary fiber intake, gram	0.043 (0.035)	0.22	0.0052 (0.011)	0.65
Alcohol intake, gram	-0.0016 (0.018)	0.93	-0.0049 (0.0060)	0.41

[1] All factors shown in the table were modeled in the same multiple linear regression. Participants in the vitamin E + selenium arm and in the vitamin E + placebo arm were included.
[2] Total variability explained, R^2=18.7%.
[3] ΔAdjusted α-TOH refers to the change in free-cholesterol-adjusted α-tocopherol concentration from baseline to year 3. Total variability explained, R^2=19.5%.
[4] β was the coefficient of the association of each factor with the change in α-tocopherol phenotype.
[5] For each categorical variable, the type III p-value indicates whether the categorical variable was significant when being included in the model, while the p-value of each categorical level (except the reference group) indicates whether the coefficient significantly differed from that of the reference group. For each continuous variable, the p-value indicates whether the coefficient significantly differed from zero.
[6] The unit for baseline α-tocopherol and γ-tocopherol concentrations is μmol/L when the outcome is the raw plasma change in α-tocopherol concentration, and the unit is μmol/mmol free cholesterol when the outcome is the plasma change in free-cholesterol-adjusted α-tocopherol concentration.

association analysis revealed no significant loci ($P < 5\times10^{-8}$) for change in tocopherol concentration (either α- or γ-TOH) in the vitE arm.

We looked up 2 SNPs reported but untested for replication in a previous GWAS of circulating α-TOH measured after 3-years of vitE supplementation among male current smokers (39). We replicated one SNP, rs2108622, which was associated with plasma α-TOH concentration after 3-years of vitE supplementation in the healthy male participants in RAS, with p-value passing the Bonferroni-corrected threshold ($P < 0.025$). Rs2108622 is a missense SNP on the cytochrome P450 family 4 subfamily F member 2 (*CYP4F2*) gene on chromosome 19. Race-specific analyses revealed consistent directions of associations for rs2108622 ($P = 0.61$ for interaction with race), thus findings with two races combined are presented herein. In the univariate model without covariate adjustment, both race and rs2108622 were associated with plasma Δα-TOH (β_{SNP} = 2.5 μmol/L, P_{SNP} = 0.0034, β_{race} = -3.0 μmol/L, P_{race} = 0.016). When the SNP and race were simultaneously modeled, the race association was borderline significant and its effect magnitude decreased by 25% (β_{SNP} = 2.1 μmol/L, P_{SNP} = 0.015, β_{race} = -2.2 μmol/L, P_{race} = 0.080) and 47.5% of the variability in plasma Δα-TOH initially explained by the race in the univariate model (R^2 = 1.0%) was explained by the SNP. In the fully adjusted model, the rs2108622-*T* minor allele (frequency = 31% in EAs and 13% in AAs) was associated with 2.4 μmol/L greater increase in plasma α-TOH after vitE supplementation ($P = 0.0032$, **Table 6**), compared to the *C* major allele. This effect magnitude is ~20% of 1 SD of plasma Δα-TOH in the vitE arm (mean change was +12.5 μmol/L). A consistent effect direction was observed in models of free-cholesterol-adjusted Δα-TOH, but findings were not statistically significant ($P = 0.33$). There was no evidence for SNP × smoking interaction ($P = 0.65$). The fully adjusted model explained 18.3% of the variability (R^2) in plasma Δα-TOH in the vitE arm ($n = 555$) and baseline concentrations of

Table 6. The association of rs2108622 with plasma change in α-tocopherol for male participants randomized to the vitamin E only arm (400 IU/day *all rac-α*-tocopherol) in the Respiratory Ancillary Study (*n* = 555)

	ΔRaw α-TOH		**ΔAdjusted α-TOH [1]**	
	β (SE) [2]	P-value [3]	β (SE) [2]	P-value [3]
Unadjusted model				
Overall (*n* = 555)	2.46 (0.83)	**0.0034**	0.48 (0.28)	0.080
- EA (***n*** = 417)	2.27 (0.93)	**0.015**	0.34 (0.30)	0.25
- AA (***n*** = 138)	1.02 (2.25)	0.65	0.30 (0.80)	0.70
Adjusted model [4]				
Overall (*n* = 555) [5]	2.36 (0.80)	**0.0032**	0.25 (0.26)	0.33
- EA (***n*** = 417)	2.42 (0.87)	**0.0058**	0.23 (0.28)	0.40
- AA (***n*** = 138)	1.42 (2.05)	0.49	0.43 (0.74)	0.56

[1] ΔAdjusted α-TOH refers to the change in free-cholesterol-adjusted α-tocopherol concentration from baseline to year 3.
[2] β was the coefficient of the association of rs2108622 with the change in α-tocopherol phenotype.
[3] P-value of the association coefficient. Significant $P < 0.05$ is in bold.
[4] Adjusted for age at baseline, BMI at baseline, race, smoking status, plasma selenium concentration, total vitamin C intake, dietary fiber intake, alcohol intake, baseline α-tocopherol, baseline γ-tocopherol, and baseline free cholesterol (only in the model with raw α-tocopherol).
[5] Total variability explained, R^2=18.3% and 19.2% for raw change in α-TOH and change in free-cholesterol-adjusted α-TOH, respectively.
Abbreviations: AA, African ancestry; EA, European ancestry.

α-TOH, γ-TOH, free cholesterol, race, and rs2108622-*T* accounted for 9.2%, 1.7%, 1.3%, 1.7%, and 1.3% of the variability, respectively (**Table 6**).

We also investigated gene-based analysis for the associations of 42 vitE-related genes on plasma Δα-TOH using MAGMA, but none of the genes reached the Bonferroni-corrected p-value threshold ($P = 0.0012$). The *CYP4F2* gene was among the 42 candidate genes related to vitE metabolism, but it did not pass nominal significance for either race ($P_{EA} = 0.13$, $P_{AA} = 0.69$).

Discussion

In a randomized clinical trial that included healthy males ≥ 50 years of age, we found that an increase in plasma α-TOH with intervention was associated with attenuated decline in pulmonary function. Moreover, the effect was strongest in participants who were adherent and participants with a positive plasma Δα-TOH (i.e., Δα-TOH > 0). Smoking modified the association such that the beneficial effect of vitE on pulmonary function decline was significant in never smokers, borderline significant in current smokers, but not in former smokers. Factors that were associated with plasma Δα-TOH included plasma α-TOH, γ-TOH, and free cholesterol all measured at study baseline, as well as race. Moreover, in replicating a previously reported association of the minor allele (*T*) of rs2108622 on *CYP4F2* with greater plasma Δα-TOH (39), we found that the allele carrier status partly explained the association of race with plasma Δα-TOH. In fully adjusted models, rs2108622 explained variability in plasma Δα-TOH equivalent to the variability explained by plasma free cholesterol, which is one known predictor of plasma α-TOH.

The Relation of Plasma Response to Supplementation and Pulmonary Function

The intent-to-treat analysis in RAS supported a beneficial effect of vitE supplementation on longitudinal pulmonary function, as the mean rate of decline in

FEV_1 in the vitE arm was -33 mL/year compared to -39 mL/year in the placebo arm (28). Although the P-value ($P = 0.19$) in the previous report (28) did not reach pre-set significance thresholds, the early stopping of SELECT led to fewer endpoint evaluations than originally planned in RAS, and lower power to detect treatment effects. To account for possible inter-individual variability in plasma response as well as non-compliance, we directly tested the association of change in plasma tocopherol biomarkers in relation to pulmonary function decline and found a protective association between plasma Δα-TOH and FEV_1 decline in the as-treated analysis, with stronger effects confirmed in sensitivity analyses.

To the best of our knowledge, this study is the first to investigate change in the vitE biomarkers on change in pulmonary function. The only prior longitudinal study of α-TOH biomarker investigated the association of vitE concentration at study baseline with subsequent change in pulmonary function and found that lower serum vitE concentration at baseline predicted a steeper FEV_1 decline in heavy smokers only (27). No prior longitudinal studies tested the γ-TOH biomarker and 2 cross-sectional studies of γ-TOH reported negative associations of γ-TOH with pulmonary function (9, 15), which was not confirmed in the RAS.

Smoking modified the association of plasma Δα-TOH with pulmonary function decline. We observed that the α-TOH concentration at study baseline was lower in current smokers (19.6 μmol/L, $P \leq 0.0004$) compared to former and never smokers (22.5 and 22.4 μmol/L, respectively). While prior studies reported that current smokers experienced a faster disappearance of α-TOH in blood circulation and used more α-TOH to combat oxidative stress compared to nonsmokers (49, 50), we found no difference in plasma Δα-TOH by smoking status. Although the α-TOH status (study baseline and change over time) was similar in former and never smokers, the rate of decline in FEV_1 was slightly greater in former smokers (-35 *versus* -33

mL/year). Since airway inflammation and pulmonary function decline are attenuated by smoking cessation (51), a supplementation-related increase in plasma antioxidant level in former smokers may be preferentially directed to scavenge oxygen radicals in tissues other than lung. If true, this could explain the lack of a positive effect of vitamin E on pulmonary function in former smokers. Future studies are needed to replicate the smoking-stratified results observed in this study and, if confirmed, to investigate tissue-specific α-TOH concentrations after vitE supplementation across all smoking groups so as to better understand the mechanism.

The Role of Non-Genetic Factors in Plasma Response to Supplementation

Previous studies investigating the determinants of plasma α-TOH were mostly cross-sectional (52-62) or based on interventions with small numbers of participants (n < 50) (63, 64). The present study is unique in considering longitudinal Δα-TOH concentration after vitE supplementation in a relatively large number of participants (*n* = 1,144), which is important given inter-individual variability in plasma responses. The inverse association of α-TOH concentration at the study baseline with plasma Δα-TOH is consistent with homeostatic mechanisms operating to keep circulating α-TOH concentrations within a normal range. Given that vitE is a lipid soluble nutrient and the amount in the circulation varies with blood lipid levels, the positive association of free cholesterol concentration at the study baseline with plasma Δα-TOH is consistent with expectation. In the liver, α-TOH and γ-TOH compete for the α-tocopherol transfer protein (αTTP) to be transported into blood circulation, and the relative affinity of αTTP with γ-TOH is only 9%, compared with α-TOH (65). This is consistent with what was observed in this study that there was a negative correlation between plasma Δα-TOH and Δγ -TOH after *all rac-α*-tocopherol supplementation (Pearson r = -0.22); the plasma concentration of γ-TOH to α-TOH was about 1:6.3 at baseline compared to 1:16.5 after 3 years of supplementation. However, we did not

observe a correlation between baseline α-TOH and γ-TOH concentrations (r = -0.018), similar to the correlation seen in another study of male participants aged 30-59 (r = -0.02) (66). We also found that *higher* baseline γ-TOH was associated with *greater* plasma Δα-TOH, independent of baseline α-TOH, whereas in a prior 6-week trial of γ-TOH supplementation (67), the plasma α-TOH concentrations did not change substantially pre- and post-intervention.

We found no evidence of an association between age and BMI with plasma Δα-TOH. A prior, and much smaller, vitE intervention study reported no association between age and plasma α-tocopherol after adjustment for cholesterol (68), consistent with our findings. While cross-sectional studies (43, 61, 62, 68-72) reported mostly positive associations between age and plasma α-tocopherol (43, 61, 68-71), these associations are likely to be driven by other factors that tend to increase with age, including blood cholesterol and increased use of vitE supplements (73). In terms of BMI, findings in previous cross-sectional studies were mixed (54-56, 57 , 58, 61, 63), with a few reporting null results for the BMI-plasma α-TOH association (54-56, 61, 63).

Smoking status is hypothesized to affect change in plasma status in response to vitE supplementation given higher levels of oxidative stress (74, 75), and faster disappearance of plasma vitE (49, 50) in smokers. However, we did not observe an appreciable effect of smoking on plasma response to vitE supplementation, consistent with a small biokinetic study which reported no effect of smoking status on plasma response to 8-week vitE supplementation (76). Moreover, prior cross-sectional studies of plasma vitE and smoking also reported mixed findings (56, 57, 77-81).

None of the nutrients/dietary factors (i.e., Se, vitamin C, fiber and alcohol) included in the model was associated with plasma Δα-TOH. The treatment arm of both vitE and Se supplementation did not make a significant difference in plasma increase

in α-TOH either, compared with vitE treatment only. Baseline plasma Se and dietary vitamin C intake were associated with plasma Δα-TOH only when baseline α-TOH was not accounted for, indicating the observed significance in univariate models may be due to positive correlations of plasma Se and dietary vitamin C intake with plasma α-TOH at baseline. We found a null association of alcohol with α-TOH biomarker concentration in our study, which was consistent with 2 cross-sectional studies in EA populations (57, 72). However, two epidemiological studies showed a positive association of dietary fiber with circulating α-TOH concentration (58, 82), and one showed null association (57). In our study, we did not obverse an association of fiber with plasma Δα-TOH, consistent with the finding in a prior study of subjects aged 46-67 in Sweden (57).

The Role of Race and Genetic Variation in Plasma Response to Supplementation

Race was a significant determinant for plasma Δα-TOH in our study: EAs had a greater increase in plasma α-TOH than AAs. The greater increases in plasma α-tocopherol in response to supplementation in EAs than AAs were seen in all smoking status groups in the vitE only arm, and among former and current smokers in the vitE+Se arm (**Supplemental Table 5**). Given the consistent trend seen in the vitE arm that the mean Δα-TOH was greater in EAs than AAs, regardless of smoking status, we further examined genetic factors which may partially explain the racial difference in plasma response to vitE supplementation. In our sample of healthy males, we replicated one missense SNP, rs2108622, in the *CYP4F2* gene from a previous GWAS of plasma α-TOH concentration, measured at year 3 after vitE supplementation, in a population of male heavy smokers. The rs2108622-T association with a greater increase in plasma vitE was also found in patients with nonalcoholic fatty liver disease (83). The CYP4F2 enzyme has been shown to be the only one among the cytochrome P450 enzymes that has the ω-hydroxylase activity for vitE catabolism (e.g., α- and γ-

TOH) in the liver (84, 85). The rs2108622-T allele results in a methionine (M), instead of a valine (V), at position 433 of the CYP4F2 enzyme. Previous evidence showed that the CYP4F2 enzyme goes through faster degradation by the proteasome (86) when M I encoded at location 433. If this is true that CYP4F2 is the only enzyme which catabolizes α-TOH to a form that can be excreted in the urine, and having a rs2108622-T minor allele would lead to fewer available CYP4F2 enzymes in the liver, we may postulate that this can result in more α-TOH in the liver available for α-TTP to be transported out into the blood circulation, leading to an increase in plasma α-TOH. Consistent with this proposed mechanism, we observed in our study that the EA population, who has a higher frequency of rs2108622-T minor allele (31% *versus* 13% in AA), had a greater increase in plasma α-TOH after supplementation.

We also observed that about half of the variability in plasma response to vitE supplementation explained by race could be explained by rs2108622. In addition, the SNP effect was not modified by race, as it was confirmed through testing the interaction of SNP by race on Δα-TOH ($P_{interaction} = 0.61$, data not shown). The direction of rs2108622-T association with plasma vitE was consistent in the two α-TOH phenotypes used (raw or free-cholesterol-adjusted), although it did not reach statistical significance for free-cholesterol-adjusted Δα-TOH. In addition, the finding of rs2108622 on plasma response to vitE supplementation was unlikely to be due to cholesterol (the ratio of free cholesterol to total cholesterol was fairly constant (42)), given that the mean free cholesterol concentration did not change substantially over the 3-year supplementation (mean free cholesterol change = -17 μmol/L, mean baseline free cholesterol = 2436 μmol/L), and that rs2108622 was not associated with change in free cholesterol ($P = 0.25$, data not shown).

The rs2108622-T on *CYP4F2* was previously reported to be associated with reduced metabolism of vitamin K_1 and thus higher hepatic level of vitamin K_1. It has

been suggested for people with rs2108622-T allele that their warfarin dose for anticoagulation purpose should be higher given the blood clotting effect of vitamin K (87). Similarly, regarding vitE metabolism, individuals with rs2108622-C major allele may need to take more vitE supplementation, compared with individuals with rs2108622-T allele, to achieve the same amount of increase in plasma α-TOH. This finding may also help identify people who are more or less likely to respond to vitE supplementation. However, based on the previous SELECT finding (29) that vitE supplementation could increase risk of prostate cancer in healthy men, caution on dose and duration is needed when taking vitE supplements, which points to the importance of precision nutrition.

The *CYP4F2* gene was included in our MAGMA gene analysis (46) using the FUMA tool (47) with a p-value of 0.13 for EAs and 0.69 for AAs, with the p-value closer to nominal significance for EAs. However, since the gene-level p-value was aggregated from SNP p-values on the gene without taking the SNP effect direction and magnitude into account, the gene-based p-value may not be a good sensitivity indicator. Future studies of gene-based analysis of plasma response to vitE supplementation should consider including the effect sizes of SNPs to estimate the effect of each gene on plasma response.

Study Strengths and Limitations

There are several strengths of this study. Through as-treated analysis of true biological Δα-TOH concentration, we observed a protective role of vitE supplementation against FEV_1 decline, whose relationship with longitudinal pulmonary function was suggestive but not statistically significant in the previous intent-to-treat analysis of the intervention assignment with FEV_1 decline (28). In addition, the sharpened estimate of increased α-TOH with attenuation in FEV_1 decline in the sensitivity analysis among adherent participants in the vitE arms further

strengthened the positive finding. To our knowledge, this is the first study to examine the longitudinal change in plasma vitE with longitudinal pulmonary function. It is also the first study to examine both non-genetic and genetic factors related to plasma response to vitE supplementation in a general healthy population, with valid and reproducible measurement of plasma tocopherol concentration at 2 time points (baseline and year 3, **Supplemental methods** for more details) and pulmonary function at multiple time points (ranged from 1 to 5 times for each individual).

There are also some limitations in this study. First, for the genetic analysis, genotype data are available only among participants in the vitE only arm (n = 555), of which the sample size afforded limited statistical power to detect statistically significant associations at the standard genome-wide threshold ($P < 5\times10^{-8}$). However, we were able to replicate one missense SNP (rs2108622) found in a previous GWAS, surpassing our Bonferroni-corrected p-value threshold for testing two candidate SNPs ($P < 0.025$). Given there are over 35,000 participants in the entire SELECT study and one-fourth were assigned to the vitamin E only arm ($n \approx 8750$), an extension of genotyping to the entire vitE arm in the SELECT trial would help increase the study's statistical power. Second, some non-genetic factors identified in the prior literature related to plasma vitE level were not measured in the study including plasma triglyceride level, other blood antioxidants such as retinol and carotenoids, and anthropometric markers such as waist circumference (WC) and waist-to-hip ratio (WHR). However, given the high correlations of triglyceride with cholesterol (r = 0.55) (88), WC and WHR with BMI ($r > 0.60$) (89, 90), and low correlations of blood antioxidants (e.g. retinol and carotenoids) with vitamin C ($0 < r \leq 0.20$) (16), we would not expect the total variability to be increased substantially when additionally accounting for these unmeasured factors. Third, when measuring the free cholesterol concentrations in RAS, there was a loss of sensitivity of the gas

chromatography column during the course of running the assays, which resulted in linearly inflated concentrations for free cholesterol. This loss of sensitivity was addressed by reconstituting, re-derivatizing, and rerunning several samples across a range of α-tocopherol and cholesterol concentrations for each day that was affected by the loss of sensitivity and correction factors were applied to all affected samples. However, given the consistent findings observed across the 2 α-TOH phenotypes (raw or free-cholesterol-adjusted) for the association of Δα-TOH with FEV_1 decline as well as for the non-genetic and genetic factors for the plasma Δα-TOH, the impact of the loss of sensitivity for the cholesterol measurement should be minor. Fourth, because the participants at RAS baseline were overall healthy males over 50 years old, the generalizability of the study could be limited and whether the same findings can be applied to the female population or other races beyond EAs and AAs is worth further investigation.

Conclusions

In terms of future work, genetic factors related to plasma response to Se supplementation as well as genetic factors that contribute to the synergistic effects of Se with vitE need further investigation to better understand the efficacy of nutrient supplementation given the difference in individual genetic makeup. The greater increase in α-TOH observed in AA never smokers in the vitE+Se arm (n = 32, **Supplemental Table 5**), compared with the rest of the vitE+Se arm was unexpected (mean = 11.6 *versus* 7.1 μmol/L), such that the synergistic effect of vitE with Se and its interaction with race and smoking are worth further exploration in the future in a larger study. In addition, if more functional SNPs related to change in plasma vitE after supplementation are identified, Mendelian Randomization analysis could be performed to further testify the causal relationship of plasma change in vitE with pulmonary function decline in cohort studies through use of genetic instruments as the

exposure (i.e., for vitE), with the advantage of genetic factors being less likely to be related to common confounding factors in conventional epidemiology studies, such as socioeconomic and lifestyle factors (91).

To summarize, we observed an association of increased plasma α-TOH with attenuation in annual FEV_1 decline after 3-year vitE supplementation in a healthy male population over 50 years old, with evidence of smoking modification. The non-genetic determinants for Δα-TOH after vitE supplementation included baseline concentrations of α-TOH, γ-TOH, cholesterol, and race. One missense variant, rs2108622 on *CYP4F2* gene, was identified to be associated with plasma Δα-TOH, which could partially explain the racial difference in plasma response to vitE supplementation. This genetic finding contributes to the evidence base for personalized nutrition by suggesting that for individuals with a rs2108622-T minor allele, their vitE intake requirement may be lower than those with a C major allele to maintain a healthy lung status.

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Supplemental Methods

Vitamin E

Samples were assayed by the same technician blinded to the sample identity and study arm, with a control sample assayed in the first and last run every day; assays were completed over a 6-month period beginning in June 2010. Both the tocopherols and free cholesterol (as silyl ethers) concentrations were quantitated against the internal standard of d9-α-tocopherol, with cholesterol adjusted for differences in detector responses. All samples were assayed in batches of eight samples (four paired samples for each individual at baseline and year 3) with one duplicate sample for one individual per batch, which generated a total of 321 duplicated sets. For all the plasma samples, the mean within-batch coefficient of variation (CV) was 9.6% for total α-tocopherol and 1.7% for free cholesterol-adjusted α-tocopherol, whereas the CV for γ-tocopherol was 13.4%, which suggested a greater variation in γ-tocopherol relative to its mean, compared to α-tocopherol. For the control samples, the mean between-run CV was 13.9% for α-tocopherol and 12.9% for cholesterol-adjusted α-tocopherol with standard deviation of 6.5 μmol/L and 2.1 μmol/mmol free cholesterol for α-tocopherol and free-cholesterol-adjusted α-tocopherol, respectively.

Genotyping

Under the additive genetic model, the minor allele was coded as 0, 1, 2 in this study. The replicated SNP, rs2108622, was directly genotyped on the MEGA^{ex} array, not based on imputation. In statistical models, the SNP was also tested as a categorical variable (0 as major allele homozygote, 1 as heterozygote, and 2 as minor allele homozygote). Having participants homozygous for the major allele as the reference group, the coefficient for participants with heterozygotes on the change in α-tocopherol outcome was +2.0 μmol/L. In comparison, in the additive model, the coefficient for participants having one additional minor allele was +2.5 μmol/L. Given

the association of SNP as a categorical variable with change in plasma vitamin E (vitE) did not differ substantially from the results under an additive genetic model, we decided to keep the additive genetic model for the main analysis.

Non-Genetic Factors in Relation to Plasma Response to Supplementation

Previous literature was searched to identify biologically plausible associations of other nutrients with vitE. Plasma selenium concentration at baseline was included given its role in reducing lipid hydroperoxides and its synergistic effect with vitE (1).

For vitamin C (vitC), which can regenerate vitE from the reaction with lipid hydroperoxyl radicals (2), it would have been ideal to include a biomarker in the statistical models. However, our study did not measure plasma vitC concentration and thus the dietary intake of vitC was used as a proxy for vitC status in this study; a prior meta-analysis reported a moderate correlation between dietary and plasma vitC (r = 0.46) in males (3), which provided a strong rationale for this approach. Alcohol was also included as a potential factor that might affect plasma vitE status, given the antioxidant effect of vitE to alleviate the oxidative stress induced by alcohol in the liver (4). For dietary fiber, there is mechanistic evidence that fiber binds to vitE in the intestine and therefore vitE absorption may be reduced in the presence of a diet higher in fiber (5, 6).

Supplemental Table 1: Vitamin E–related genes

Names of Vitamin E Metabolic Genes (Abbreviation)	Chromosomal Location	Gene Length (bp)
ATP-binding Cassette, Subfamily A [ABC1], Member 1 (ABCA1)	9q31.1	147152
ATP-binding Cassette, Subfamily B [MDR/TAP], Member 1 (ABCB1)	7q21.12	209616
ATP-binding Cassette, Subfamily B [MDR/TAP], Member 4 (ABCB4)	7q21.1	73658
ATP-binding Cassette, Subfamily C [CFTR/MRP], Member 2 (ABCC2)	10q24.2	69199
ATP-binding Cassette, Subfamily G, Member 1 (ABCG1)	21q22.3	104701
Afamin (AFM)	4q13.3	22257
Apolipoprotein A-I (APOA1)	11q23-q24	1897
Apolipoprotein A-IV (APOA4)	11q23.3	2593
Apolipoprotein A-V (APOA5)	11q23.3	3050
Apolipoprotein B [including Ag(x) antigen] (APOB)	2p24.1	42644
Apolipoprotein B Receptor (APOBR)	16p12.1	4322
Apolipoprotein C-III (APOC3)	11q23.3	3163
Apolipoprotein E (APOE)	19q13.32	3611
CD36 Molecule [thrombospondin receptor] (CD36)	7q21.11	76000
Carboxyl Ester Lipase (CEL)	9q34.13	9886
Cholesteryl Ester Transfer Protein, Plasma (CETP)	16q13	21921
Cytochrome P450, Family 3, Subfamily A, Polypeptide 4 (CYP3A4)	7q22.1	27204
Cytochrome P450, Family 3, Subfamily A, Polypeptide 5 (CYP3A5)	7q22.1	87004
Cytochrome P450, Family 4, Subfamily F, Polypeptide 2 (CYP4F2)	19p13.12	20050
Cytochrome P450, Family 4, Subfamily F, Member 11 (CYP4F11)	19p13.1	22497
Fatty Acid Binding Protein 2, Intestinal (FABP2)	4q26	3274
Glutathione S-transferase Omega 1 (GSTO1)	10q25.1	30545
Glutathione S-transferase Omega 2 (GSTO2)	10q25.1	16535
Haptoglobin (HP)	16q22.2	6447
Low Density Lipoprotein Receptor (LDLR)	19p13.2	44448
Lipase, Hepatic (LIPC)	15q21.3	136880
Lipase F, Gastric Type (LIPF)	10q23.31	14427
Lipoprotein Lipase (LPL)	8p21.3	28188

Names of Vitamin E Metabolic Genes (Abbreviation)	Chromosomal Location	Gene Length (bp)
Low Density Lipoprotein Receptor-related Protein 1 (LRP1)	12q13.3	84843
Microsomal Triglyceride Transfer Protein (MTTP)	4q23	49188
NPC1 [Niemann-Pick disease, type C1, gene]-like 1 (NPC1L1)	7p13	28779
Phospholipid transfer protein (PLTP)	20q13.12	13745
Pancreatic Lipase (PNLIP)	10q25.3	21940
Scavenger Receptor Class B, Member 1 (SCARB1)	12q24.31	86345
Solute Carrier Family 2 [facilitated glucose transporter], Member 1 (SLC2A1)	1p34.2	33801
Solute Carrier Family 2 [facilitated glucose transporter], Member 3 (SLC2A3)	12p13.31	17045
Solute Carrier Family 10, Member 2 (SLC10A2 or ASBT)	13q33.1	22849
Sodium-coupled Vitamin C Transporters 1 (SLC23A1)	5q31.2	17358
Sodium-coupled Vitamin C Transporters 2 (SLC23A2)	20p13	157938
Tocopherol-associated protein 3 (SEC14L4 or TAP3)	22q12.2	16822
SEC14-like Lipid Binding 2 (SEC14L2)	22q12.2	28363
Tocopherol [alpha] Transfer Protein (TTPA)	8q12.3	25181

Supplemental Table 2. Association of plasma change in γ-tocopherol after 3-year vitamin E supplementation with annual rate of change in FEV_1 (*n* = 1,144)

	Full sample [1] (*n* = 1,144)			**Sample with increased α-TOH [1,2] (*n* = 857)**			**Adherent sample with increased α-TOH [1,2,3] (*n* = 609)**		
	Unadjusted β (SE) [4]	Adjusted β (SE) [4,5]	P [6]	Unadjusted β (SE) [4]	Adjusted β (SE) [4,5]	P [6]	Unadjusted β (SE) [4]	Adjusted β (SE) [4,5]	P [6]
ΔRaw γ-TOH	-0.39 (1.05)	-0.52 (1.06)	0.62	0.57 (1.24)	0.42 (1.25)	0.74	0.25 (1.48)	0.19 (1.50)	0.90
ΔAdjusted γ-TOH [7]	-5.20 (2.95)	-5.46 (2.97)	0.067	-4.29 (3.62)	-4.59 (3.65)	0.21	-6.50 (4.34)	-6.53 (4.38)	0.14

[1] All participants in the vitamin E + selenium arm and in the vitamin E + placebo arm.
[2] Positive change in plasma α-tocopherol concentration after 3 years of *all rac-α*-tocopherol supplementation.
[3] Adherent participants (defined as self-report of taking at least 80% of the supplement pills) during the 3 years of supplementation.
[4] β was the coefficient of the Δγ-TOH × time term in the statistical model.
[5] Adjusted for age at baseline, height, race, smoking status, smoking dose at baseline, treatment arm, baseline α-tocopherol, baseline γ-tocopherol, and baseline free cholesterol (only in the model with raw γ-tocopherol).
[6] P-value of the Δγ-TOH × time term in the model adjusted for other covariates.
[7] ΔAdjusted γ-TOH refers to the change in free-cholesterol-adjusted γ-tocopherol concentration from baseline to year 3.

Supplemental Table 3. Interaction of plasma change in α-tocopherol after 3-year vitamin E supplementation with smoking status on annual rate of change in FEV_1 in the two vitamin E arms (*n* = 1,144) [1]

	ΔRaw α-TOH		ΔAdjusted α-TOH [2]	
	β (SE)	P [3]	β (SE)	P [3]
Smoking status		**Type III = 0.013**		**Type III = 0.032**
- **Current smokers [4]**	ref - 0.09 (0.59)	0.87	ref + 0.32 (1.86)	0.86
- **Former smokers [4]**	ref - 1.23 (0.46)	**0.0069**	ref – 3.07 (1.33)	**0.021**
- **Never smokers (ref) [5]**	0.83 (0.35)	**0.017**	2.43 (1.01)	**0.017**

[1] All participants in the vitamin E + selenium arm and in the vitamin E + placebo arm. The model has been adjusted for age at baseline, height, race, smoking status, smoking dose at baseline, treatment arm, baseline α-tocopherol, baseline γ-tocopherol, and baseline free cholesterol (only in the model with raw α-tocopherol).
[2] ΔAdjusted α-TOH refers to the change in free-cholesterol-adjusted α-tocopherol concentration from baseline to year 3.
[3] The type III p-value was based on the 3-way interaction term (Δα-TOH × time × smoking status) that whether this interaction term was significant when included in the model. The p-value for never smokers indicates whether the coefficient significantly differed from zero, while the p-value for current/former smokers indicates whether the coefficient significantly differed from that of never smokers.
[4] β was the coefficient of the 3-way interaction term (Δα-TOH × time × smoking status) in the statistical model.
[5] β was the coefficient of the Δα-TOH × time term in the statistical model.

Supplemental Table 4. Association of plasma change in α-tocopherol after 3-year vitamin E supplementation with annual rate of change in FEV_1 among adherent participants in the two vitamin E arms, stratified by smoking status (*n* = 776) [1]

	ΔRaw α-TOH		ΔAdjusted α-TOH [2]	
	β (SE) [3]	P [4]	β (SE) [3]	P [4]
Smoking status				
- Never smokers (*n* = 242)	0.72 (0.44)	0.10	2.89 (1.34)	**0.032**
- Former smokers (*n* = 420)	-0.34 (0.35)	0.33	-0.31 (1.07)	0.77
- Current smokers (*n* = 114)	1.00 (0.68)	0.15	3.04 (2.13)	0.16

[1] All adherent participants (defined as self-report of taking at least 80% of the supplement pills) in the vitamin E + selenium arm and in the vitamin E + placebo arm. The model has been adjusted for age at baseline, height, race, smoking status, smoking dose at baseline, treatment arm, baseline α-tocopherol, baseline γ-tocopherol, and baseline free cholesterol (only in the model with raw α-tocopherol).

[2] ΔAdjusted α-TOH refers to the change in free-cholesterol-adjusted α-tocopherol concentration from baseline to year 3.

[3] β was the coefficient of the Δα-TOH × time term in the statistical model for each smoking stratum.

[4] P-value of the Δα-TOH × time term in the model adjusted for other covariates. Significant $P < 0.05$ is in bold.

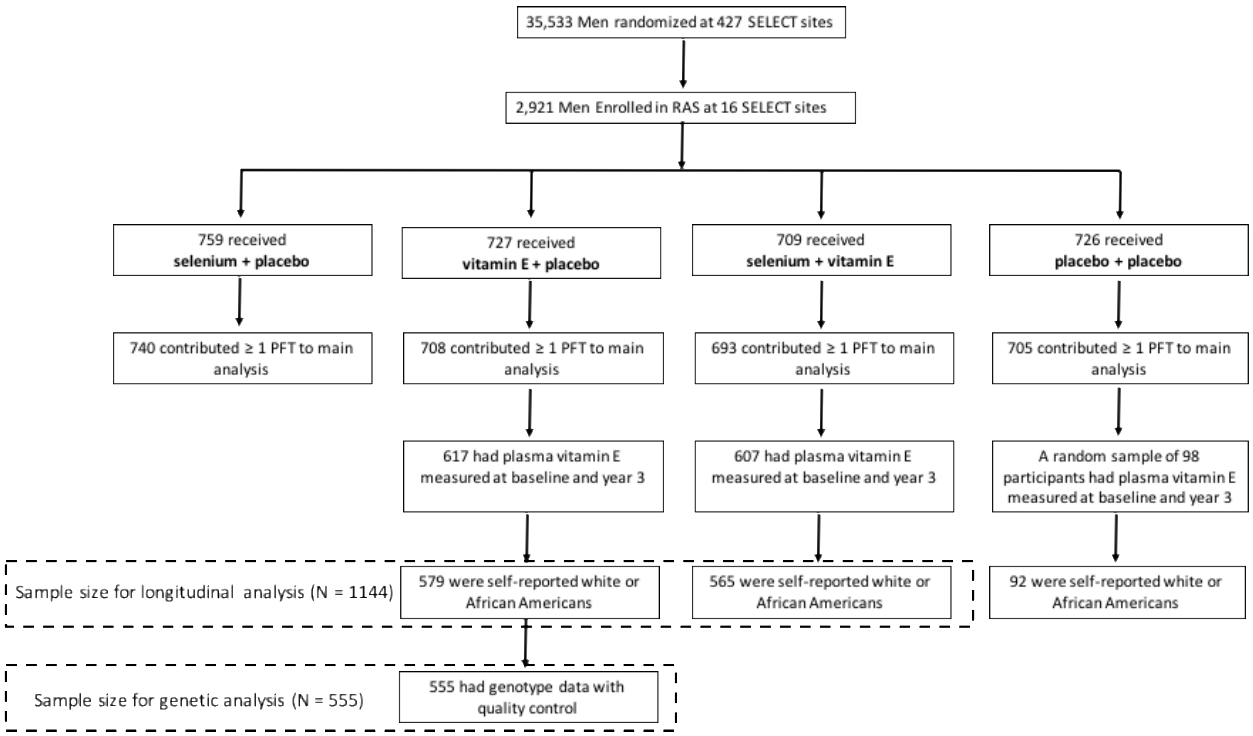
Supplemental Table 5. Plasma change in α-tocopherol by cigarette smoking status in the two vitamin E arms in the Respiratory Ancillary Study (*n* = 1,144) [1]

	ΔRaw α-TOH (μmol/L)					
	EA participants in the vitE arm (*n* = 439)	AA participants in the vitE arm (*n* = 140)	$P_{EA\ vs\ AA}$ [2]	EA participants in the vitE+Se arm (*n* = 435)	AA participants in the vitE+Se arm (*n* = 130)	$P_{EA\ vs\ AA}$ [2]
Never smoker	7.8 (12.9)	6.5 (11.2)	0.58	7.7 (14.6)	11.6 (12.0)	0.17
Former smoker	9.5 (12.3)	4.7 (13.7)	**0.0087**	7.3 (15.9)	5.3 (10.3)	0.28
Current smoker	10.1 (12.5)	7.0 (10.6)	0.17	8.0 (13.5)	5.4 (12.9)	0.31

[1] Data are presented as mean (standard deviation) in the table, unless otherwise noted. Change in tocopherol concentration was calculated as tocopherol concentration at baseline (or pre-supplementation) subtracted from that at year 3; positive values represent increases in tocopherol from baseline to year three.

[2] P value is the significance level for difference in mean plasma change in α-TOH across EA and AA participants. Significant $P < 0.05$ is in bold.

Abbreviations: AA, African ancestry; EA, European ancestry; vitE, vitamin E supplementation only arm; vitE+Se, vitamin E + selenium supplementation arm.



Supplemental Figure 1. Flowchart of sample size included in this study

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CHAPTER 5

CONCLUSION

Overview

The overarching objective of this dissertation was to investigate gene-by-nutrient interactions to better understand the role of nutrients with anti-inflammatory and anti-oxidative properties in pulmonary function. Ultimately, the findings from this research are expected to contribute to novel intervention approaches to mitigate steep decline in pulmonary function and to prevent risk of chronic obstructive pulmonary disease (COPD).

The first project studied the association of a vitamin D biomarker, serum 25(OH)D, with pulmonary function. The serum 25(OH)D—pulmonary function cross-sectional association was estimated in eight racially-diverse population-based cohort studies in the CHARGE Consortium, which was novel because few studies investigate this association in adults of ancestry groups other than European (1). Cross-ancestry meta-analysis found serum 25(OH)D had a positive association with FEV_1 and FVC, with effect magnitude and direction consistent across cohorts and across European (EA) and African ancestries (AA). Smoking modified the association, and there was a 2-fold stronger association in current and former smokers, compared with never smokers. While the cross-sectional design limits causal inference, the large sample size (22,838 EA and 4,290 AA), the inclusion of diverse U.S. and European cohorts (i.e., Netherlands, Iceland), and the highly consistent associations across 8 cohorts strengthens the plausibility of a causal link. Further longitudinal studies are needed to investigate whether a *higher* level of serum vitamin D leads to an *attenuated rate of decline* in pulmonary function.

In the first stage of the second project, we investigated the association of omega-3 fatty acid biomarkers with pulmonary function. In the second stage, we used a gene-by-environment design (i.e., genome-wide interaction study, GWIS) to identify novel genetic variant—pulmonary function associations that varied by omega-3 fatty acid status. These questions were investigated in seven racially-diverse cohort studies in the CHARGE Consortium. We found positive associations of DHA and DPA with FEV_1 and FVC, with an effect size for the DHA-FEV_1 association that was 3-fold greater in current smokers compared to never and former smokers. Thus, current smokers who were 1% higher on the DHA biomarker had a 39mL higher FEV_1. In the second stage, we followed up the positive associations of DHA and DPA on pulmonary function with a GWIS to identify genetic variant(s)—pulmonary function associations that varied by the omega-3 fatty acid biomarker concentration. A novel intronic *DPP10* SNP, rs11693320, was identified ($P = 4.5\times10^{-8}$) and replicated ($P = 0.045$) in an independent cohort study. Overall, participants with the rs11693320 major allele-A had an FVC that was 161-mL *lower*. However, in participants with a 1% *higher* DHA concentration, the rs11693320 major allele-A association with FVC was *attenuated* by 36.2 mL; thus about 22% of the negative SNP effect was mitigated in persons with higher omega 3 fatty acid status. The identification of a novel rs11693320—DHA interaction demonstrates that the effect of genetic variants on the pulmonary phenotype may vary by nutritional status. This finding supports a proposed causal role for the omega 3 fatty acids in pulmonary health, and ultimately, this and other GWIS findings are expected to contribute to the information needed for providing targeted dietary advice to population subgroups.

Finally, previous GWAS of nutrient biomarkers reveal the heritability of nutritional status (2-4). A better understanding of the genetic architecture underlying nutritional status offers a novel approach to study the role of nutrition in the

development of chronic disease. In the third dissertation project, we investigated whether genetic variation was associated with the change in plasma vitamin E after supplementation. This research project used data from a previously conducted randomized controlled trial of vitamin E and selenium supplementation in a healthy male population aged 50 years and older. First, we conducted an as-treated analysis and found that a *larger* plasma increase in vitamin E, specifically in α-tocopherol, was associated with a *greater* subsequent attenuation of FEV_1 decline, especially in never and current smokers. In participants adherent to supplementation, a 1 standard deviation *higher* change in α-tocopherol (i.e., 4 μmol/mmol free cholesterol α-tocopherol) was associated with an attenuation of ~9mL/year in the FEV_1 rate of decline; given the annual FEV_1 rate of decline is about 35mL/year in this population, this effect size corresponds to an attenuation of about ~25% of the effect of 1 year of aging. Next, we investigated both the genetic and the non-genetic factors associated with the change in plasma vitamin E after supplementation. On average, participants with greater increase in plasma vitamin E in response to supplementation had a *lower* baseline level of plasma α-tocopherol, a *higher* baseline level of plasma γ-tocopherol, and a *higher* baseline level of free cholesterol. In addition, we found the increase in plasma α-tocopherol was greater in EA compared to AA participants. Our investigation of genetic variants associated with the change in plasma vitamin E after supplementation replicated a missense variant identified in a previous GWAS of heavy male smokers (5): rs2108622 on the *CYP4F2* gene, with a minor T allele frequency of 31% in the EA participants and 13% in the AA. This SNP was associated with a *greater* increase in plasma α-tocopherol in our healthy male population ($P = 0.0032$), and in further analyses this SNP accounted for 47.5% of the race contribution to the variability in plasma response to vitamin E supplementation. Overall, this project found a protective role of vitamin E supplementation in pulmonary function decline in

a healthy male population, which may provide a feasible intervention for COPD prevention. Moreover, our finding of a *CYP4F2* variant associated with greater response to supplementation contributes to the evidence that genetic subgroups may respond differentially to dietary supplementation.

Emerging Themes and Future Directions

In all my dissertation projects, we tested whether the nutrient—pulmonary function association was modified by smoking. While all persons have some oxidant burden, given smokers have an additional burden of oxidative stress due to cigarette smoke exposure, the effects of antioxidant and anti-inflammatory nutrients on pulmonary function were expected to differ by smoking status (6, 7). Across the projects comprising the dissertation, the effect modification by smoking was not consistent for the 3 nutrient biomarkers (vitamin D, omega-3 fatty acids, and vitamin E). The positive association of serum 25(OH)D with FVC was 2-fold higher in both current and former smokers compared to never smokers, with similar trends for the FEV_1 outcome. The positive association of the DHA biomarker with FEV_1 was 3-fold higher in current smokers, compared to never and former smokers. Finally, the positive association of change in plasma α-tocopherol with attenuated FEV_1 decline was found in never and current smokers, but not former smokers. In all projects, the nutrients had stronger effects in current smokers, consistent with the hypothesis that these nutrients are efficient in countering the tissue damage associated with the oxidant burden of smoking. The inconsistencies in whether the nutrients have less effect in former and/or never smokers may be explained by differences in the role of these nutrients in countering the general body burden of oxidative stress and inflammation. Differences in these associations suggest the need for further studies to better understand the role of these nutrients under different physiologic conditions,

and the inconsistent trend seen in former smokers for different nutrients warrants further investigation to understand if there is a biological mechanism to explain these observations.

For the first project, which identified an association of vitamin D with pulmonary function, we found a consistent positive relation across all cohorts and ancestries. Further studies are needed to examine whether the cross-sectional finding holds true for longitudinal pulmonary function. In addition, given known heritability in serum vitamin D (2) and the role of vitamin D in gene regulation in lung tissues (8, 9), further studies should investigate the gene-by-vitamin D interaction effects on the pulmonary function phenotype via genome-wide interaction analysis (10).

For the second project, which found a novel association of the DHA biomarker with pulmonary function and revealed a DHA-by-*DPP10* interaction, further studies of the longitudinal pulmonary function phenotype would be informative. One prior candidate gene study reported significant associations of multiple SNPs on *DPP10* with FEV_1 and FVC decline in a healthy population of white men (11). The novel SNP identified in our study is not in linkage disequilibrium with any of the significant *DPP10* SNPs reported previously, suggesting it is an independent locus. In addition, given that our smoking-stratified analysis showed a *greater* negative effect of the *DPP10* SNP on pulmonary function and a greater attenuation effect of DHA among former smokers, the potential effect of smoking on *DPP10* and its interaction with DHA also warrant further investigation.

In the Respiratory Ancillary Study (RAS) to the Selenium and Vitamin E Cancer Prevention Trial (SELECT), we found that a *greater* increase in plasma α-tocopherol in response to vitamin E supplementation was associated with an attenuation in FEV_1 decline in never and current cigarette smokers. In analyses further limited by adherence, this association was strengthened. While the intention-to-treat

analysis of vitamin E with longitudinal pulmonary function in the RAS did not reach pre-set statistical thresholds (12), the premature termination of SELECT (13) led to lowered power. However, the as-treated findings reported herein support a causal association, and these findings deserve further studies to consolidate the causal inference. For example, the findings would be strengthened by a Mendelian Randomization (MR) analysis to use genetic instruments as a confounder-free proxy for plasma vitamin E status in order to estimate the vitamin E level—pulmonary function decline association. A key advantage of using genetic instruments in the MR analysis is that exposure-related genetic variants are typically unrelated to the behavioral and lifestyle confounding that limits inference in observational epidemiologic studies (14).

Given the small sample with genotype data in RAS (N = 555 men), the genome-wide analysis of plasma change in α-tocopherol in response to supplementation had limited power to identify novel variants, although our study did provide the first replication of a variant on *CYP4F2* in a healthy male population. The future plan to genotype the ~35,000 SELECT randomized controlled trial participants will ultimately provide the sample size needed for a more definitive study of the genetic architecture of the change in vitamin E biomarkers in response to supplementation. In summary, given that the SELECT study reported that vitamin E supplementation was associated with an increased risk of prostate cancer (15), any recommendation involving vitamin E intake needs to integrate the risks and benefits to achieve the best population-level and personalized nutrition advice.

In this dissertation research, single nutrients (e.g., vitamin D, omega-3 fatty acids, vitamin E) were investigated rather than foods or overall dietary patterns. The merit of studying single nutrients is that it helps disentangle the causative factors in the diet that are directly involved in disease etiology so as to better understand the

underlying biological mechanisms (16). However, given that foods are consumed rather than nutrients, and because each food comprises various types and amounts of nutrients that may be synergistic or antagonistic in the body, studies that consider dietary patterns are needed to guide policies around dietary advice for population health (e.g., 2015-2020 Dietary Guidelines for Americans (17)). Studies that investigate how genetic variation may influence food preferences and intake as well as how overall dietary pattern influences downstream gene expression and biological pathways involved in the etiology of chronic diseases are important, and are needed to contribute to the strongest evidence base for developing targeted dietary guidelines for population subgroups.

On the global scale, there is an increasing burden of chronic diseases (18), and an estimated 11 million deaths were attributed to poor diets in 2017 (19). The role of nutrition and diet in preventing the onset and progression of chronic diseases is critically important. The innovative approaches used in this dissertation research included the integration of big data across ancestries in a cross-cohort collaboration, the investigation of gene-by-nutrient interactions on intermediate outcomes related to chronic disease risk, and the investigation of genetic contribution to nutritional status to add to the evidence needed for personalized nutrition advice. These approaches yielded novel insights that contribute to identifying nutritional interventions for chronic lung disease prevention in population subgroups. Future studies that use creative and innovative approaches to integrate biomedical big data, including genomics, metabolomics, gut microbiome, nutritional status and other environmental exposures in research and/or clinical settings, are needed to better understand the pathophysiology of chronic diseases. Such studies are expected to contribute important evidence to form the basis for personalized dietary advices to increase the healthy life span. Examples of this approach are beginning to be published, including a recent

study that demonstrated the plausibility of using personalized health information and real-time food logging to predict individual blood glucose responses for the prevention of diabetes and cardiovascular diseases (20).

In conclusion, to strengthen the causal inference in studies of the nutrition—disease link, we must accumulate evidence to support the effect of the nutrient and/or diet across diverse study types including animal studies, mechanistic studies in human cells, prospective cohort studies and, where appropriate, randomized controlled trials for both intermediate and hard disease endpoints (21). In order to translate findings from nutritional epidemiology to contribute to the formulation of national policy and guidelines, high quality meta-analyses and systematic reviews are important (21). The research presented in this dissertation was designed to address research gaps in the evidence base for chronic disease prevention by leveraging big data across multiple ancestry groups and by using novel approaches to investigate the role of nutrition in chronic diseases.

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