# 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 ($\beta_{SNP}$) 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 $1.25 \times 10^{-8}$ 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 $\beta_{SNP}$ [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

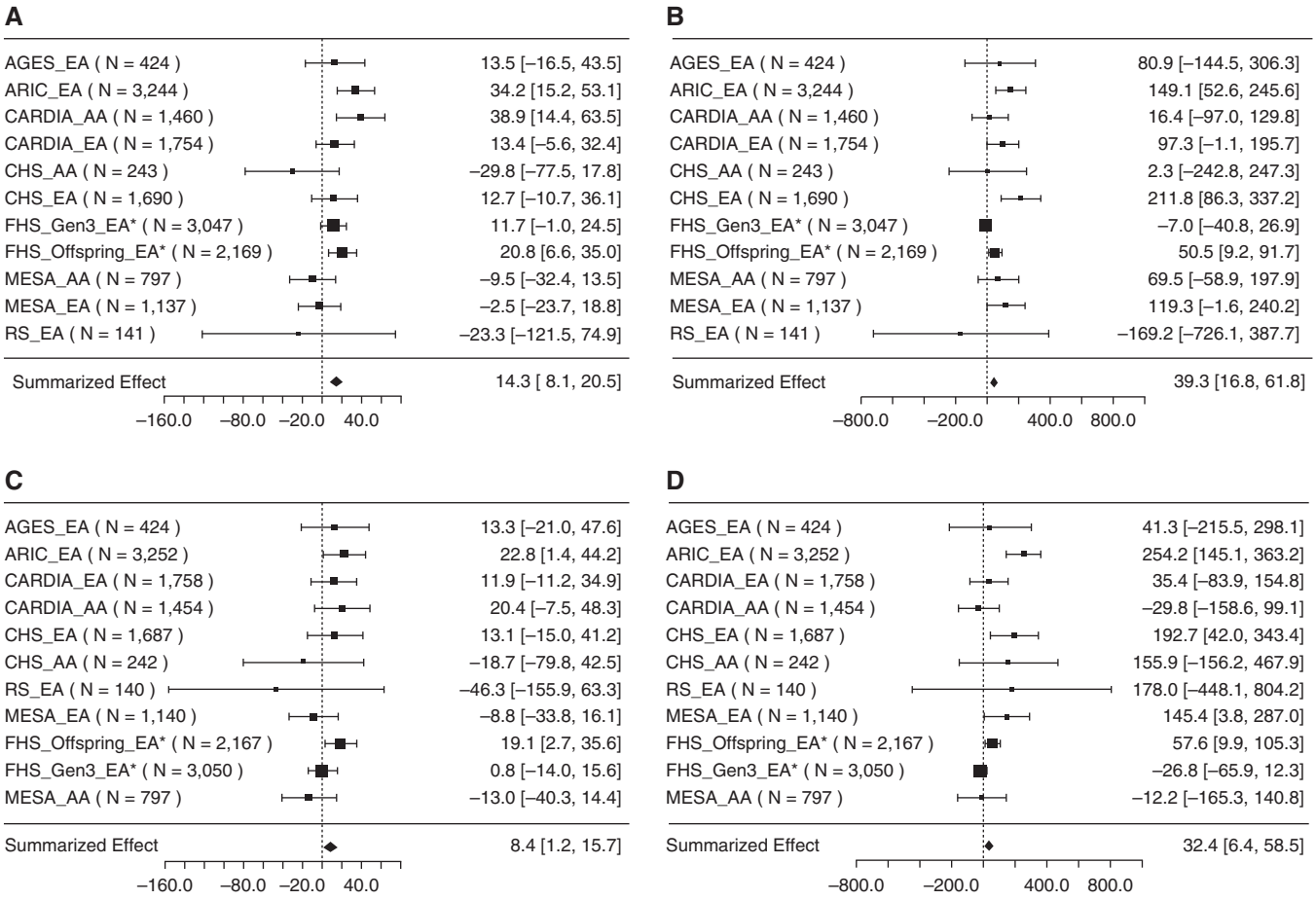


**Figure 1.** Forest plots of the meta-analysis of omega-3 fatty acid biomarkers on $FEV_1$ and FVC. Associations are presented for (*A*) docosahexaenoic acid (DHA)–$FEV_1$, (*B*) docosapentaenoic acid (DPA)–$FEV_1$, (*C*) DHA–FVC, and (*D*) DPA–FVC. β (ml) denotes the coefficient from the fixed-effects 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 $FEV_1$

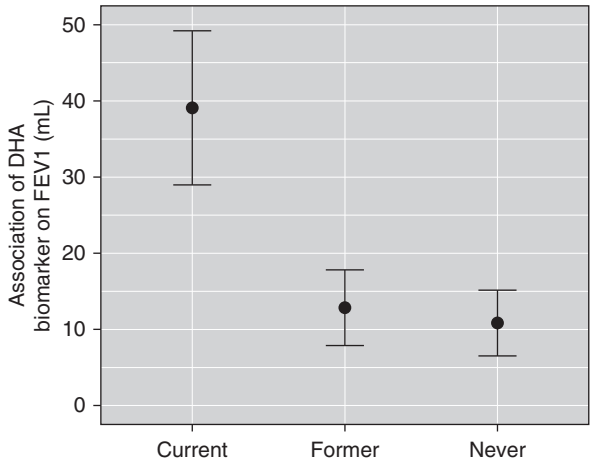


**Figure 2.** Meta-analysis of the association of DHA biomarker–$FEV_1$ outcome, by smoking status. Smoking status categories were never smoker, former smoker, and current smoker. The *y*-axis shows the coefficient β (unit: ml), which denotes that a 1% (of total fatty acids) higher DHA level was associated 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

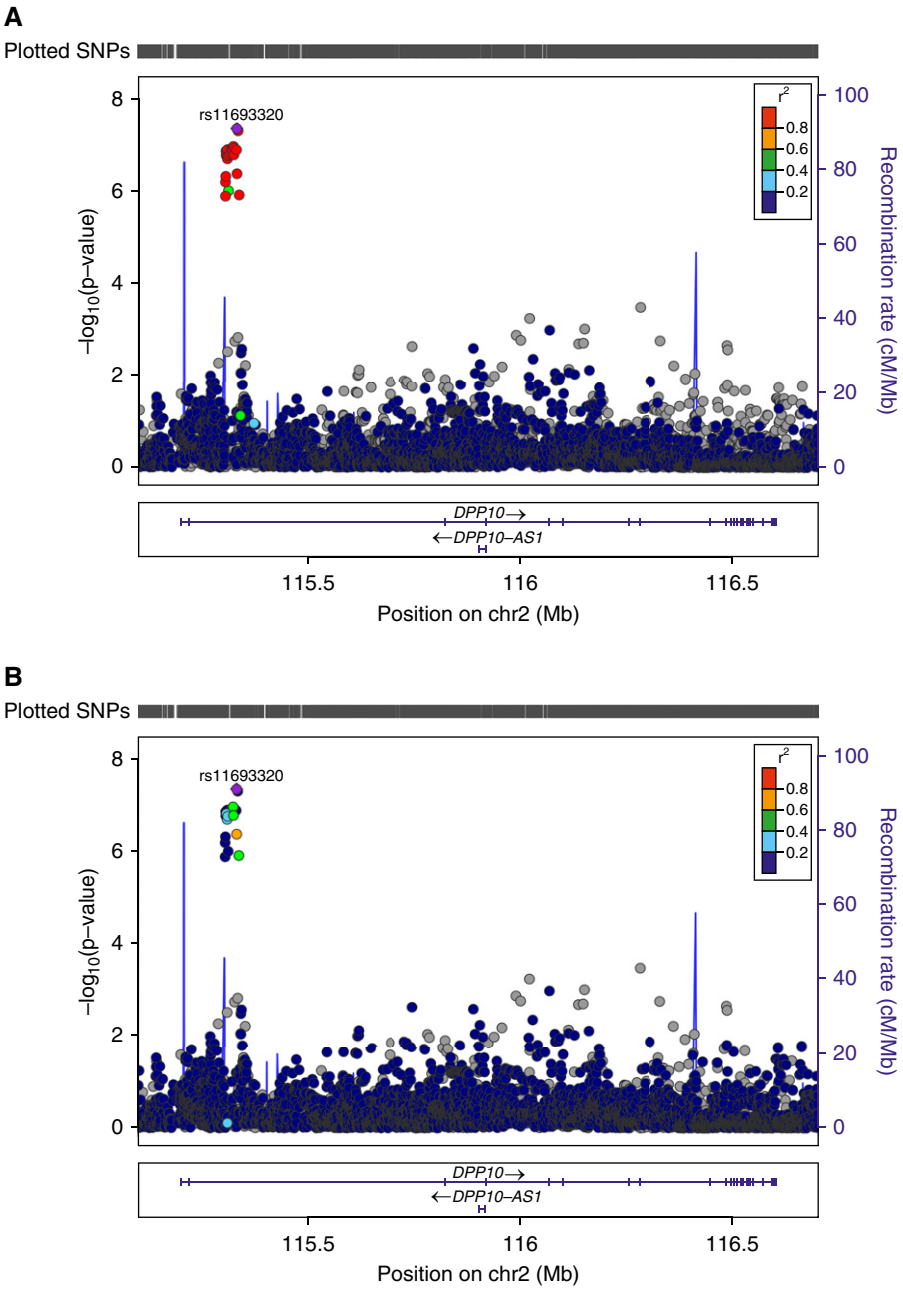


**Figure 3.** Novel *DPP10* locus identified at genome-wide significance ($P_{2df} < 5 \times 10^{-8}$) for FVC, accounting for SNP/indel × docosahexaenoic acid interaction. SNP/indel associations are shown from the cross-ancestry joint 2*df* meta-analysis across *DPP10* and its 100-kb flanking region (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

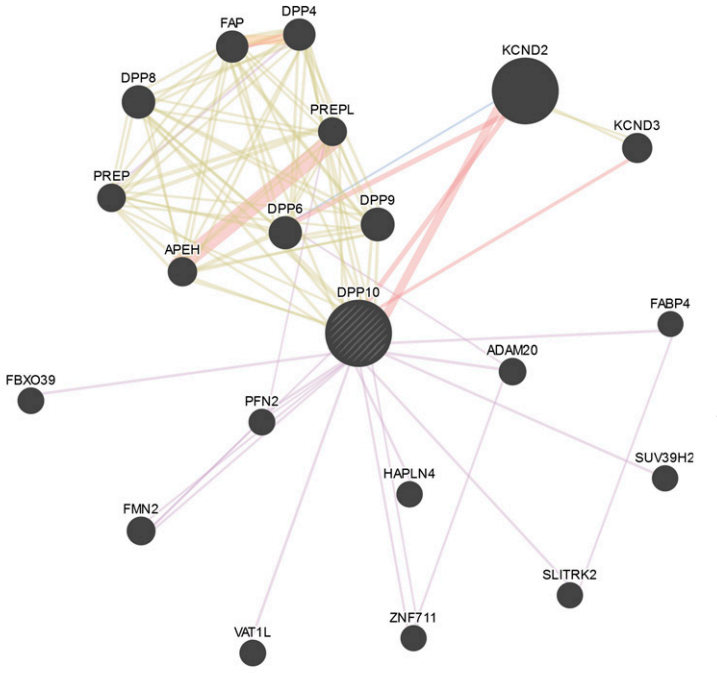
| Cohort and Ancestry (Sample Size) | A Allele Frequency | Model without Interaction with DHA (*Total N = 13,649*)* | | Model with Interaction with DHA (*Total N = 13,649*)† | | | | |
|---|---|---|---|---|---|---|---|---|
| | | β (SE) | *P* | $\beta_{SNP}$ (SE) | $P_{SNP}$ | $\beta_{SNP\times DHA\ interaction}$ (SE) | $P_{SNP\times DHA\ interaction}$ | $P_{2df}$ |
| Discovery cohorts | | | | | | | | |
| ARIC EA (*n* = 3,143) | 0.80 | −65.9 (18.0) | $2.4 \times 10^{-4}$ | −145.6 (60.0) | 0.015 | 28.3 (20.4) | 0.17 | $4.4 \times 10^{-4}$ |
| 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) | $2.7 \times 10^{-4}$ | −155.8 (28.7) | $5.8 \times 10^{-8}$ | 34.0 (7.6) | $7.4 \times 10^{-6}$ | $1.5 \times 10^{-7}$ |
| Discovery cohort meta-analysis (EA and AA) | | −34.9 (9.6) | $2.9 \times 10^{-4}$ | −157.1 (27.7) | $\mathbf{1.4 \times 10^{-8}}$ | 34.3 (7.2) | $1.7 \times 10^{-6}$ | $\mathbf{4.5 \times 10^{-8}}$ |
| 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) | $1.7 \times 10^{-4}$ | −161.0 (26.9) | $\mathbf{2.1 \times 10^{-9}}$ | 36.2 (7.0) | $2.1 \times 10^{-7}$ | $\mathbf{9.4 \times 10^{-9}}$ |

*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 | $2.4 \times 10^{-7}$ |
| **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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