Genetic loci associated with circulating phospholipid *trans* fatty acids: a meta-analysis of genome-wide association studies from the CHARGE Consortium[1–7]

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ABSTRACT

Background: Circulating *trans* fatty acids (TFAs), which cannot be synthesized by humans, are linked to adverse health outcomes. Although TFAs are obtained from diet, little is known about subsequent influences (e.g., relating to incorporation, metabolism, or intercompetition with other fatty acids) that could alter circulating concentrations and possibly modulate or mediate impacts on health.

Objective: The objective was to elucidate novel biologic pathways that may influence circulating TFAs by evaluating associations between common genetic variation and TFA biomarkers.

Design: We performed meta-analyses using 7 cohorts of European-ancestry participants (n = 8013) having measured genome-wide variation in single-nucleotide polymorphisms (SNPs) and circulating TFA biomarkers (erythrocyte or plasma phospholipids), including *trans*-16:1n–7, total *trans*-18:1, *trans*/*cis*-18:2, *cis*/*trans*-18:2, and *trans*/*trans*-18:2. We further evaluated SNPs with genome-wide significant associations among African Americans (n = 1082), Chinese Americans (n = 669), and Hispanic Americans (n = 657) from 2 of these cohorts.

Results: Among European-ancestry participants, 31 SNPs in or near the fatty acid desaturase (*FADS*) 1 and 2 cluster were associated with *cis*/*trans*-18:2; a top hit was rs174548 (β = 0.0035, $P = 4.90 \times 10^{-15}$), an SNP previously associated with circulating n–3 and n–6 polyunsaturated fatty acid concentrations. No significant association was identified for other TFAs. rs174548 in *FADS1/2* was also associated with *cis*/*trans*-18:2 in Hispanic Americans (β = 0.0053, $P = 1.05 \times 10^{-6}$) and Chinese Americans (β = 0.0028, P = 0.002) but not African Americans (β = 0.0009, P = 0.34); however, in African Americans, fine mapping identified a top hit in *FADS2* associated with *cis*/*trans*-18:2 (rs174579: β = 0.0118, $P = 4.05 \times 10^{-5}$). The association between rs174548 and *cis*/*trans*-18:2 remained significant after further adjustment for individual circulating n–3 and n–6 fatty acids, except arachidonic acid. After adjustment for arachidonic acid concentrations, the association between rs174548 and *cis*/*trans*-18:2 was nearly eliminated in European-ancestry participants (β-coefficient reduced by 86%), with similar reductions in Hispanic Americans and Chinese Americans.

Conclusions: Our findings provide novel evidence for genetic regulation of *cis*/*trans*-18:2 by the *FADS1/2* cluster and suggest that this regulation may be influenced/mediated by concentrations of arachidonic acid, an n–6 polyunsaturated fat. *Am J Clin Nutr* 2015;101:398–406.

Keywords arachidonic acid, genome-wide association, meta-analysis, phospholipid, *trans* fatty acids

INTRODUCTION

trans Fatty acids (TFAs),[8] unsaturated fatty acids with one or more double bonds in the *trans* configuration, have adverse health effects (1, 2). Concentrations of TFAs in circulating phospholipids are linked to greater systematic inflammation (3, 4), sudden cardiac death, and fatal coronary artery disease (5, 6). Because *trans* bonds cannot be synthesized by humans, exposure to TFAs is only from dietary consumption, especially from packaged or prepared foods containing partially hydrogenated vegetable oils but also from meats or milk products from ruminants (e.g., cattle, sheep, and goats) that contain small amounts of TFAs (7). However, circulating TFA concentrations are only partly correlated with dietary intakes of these foods (7), suggesting potential

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nondietary influences. Yet, following consumption, little is known about the metabolic fate of TFA or its determinants. For instance, endogenous mechanisms of incorporation, metabolism, and/or competition with other fatty acids could influence circulating TFA concentrations (8) and modulate or mediate their impact on health.

Phospholipid fatty acids, the major components of cellular membranes, influence the function of membrane-bound receptors and channels that are involved in various signal transductions and act as precursors to a myriad of active lipid metabolites (9). They also serve as biomarkers for the potential effects of individual fatty acids on health and disease. For example, phospholipid concentrations of some TFA subtypes, such as those with 16 carbons and 1 double bond at the seventh N-terminal carbon (i.e., *trans*-16:1n–7), are not associated with coronary artery disease and have been linked to lower risk of type 2 diabetes mellitus (7, 10). In contrast, phospholipid concentrations of other TFA subtypes, especially those with 18 carbons and 2 double bonds (i.e., *trans*-18:2 fatty acids), are strongly associated with inflammation and cardiac death, even more so than concentrations of *trans*-18:1 fatty acids, the most common TFA in the diet (3–6). *Trans*-18:2 fatty acids are particularly enigmatic because their circulating concentrations are associated with adverse clinical outcomes at very low concentrations, and these circulating concentrations are also not strongly correlated with dietary intakes of foods high in partially hydrogenated vegetable oils (7). The latter finding suggests that 18:2 TFAs may be influenced by metabolism or come from other industrial sources, such as vegetable oil deodorization or high-temperature frying (8).

To elucidate the biologic processes that might affect circulating phospholipid TFAs, including potentially novel metabolic pathways, we performed a collaborative investigation that pooled data from 7 prospective genome-wide association studies (GWASs) of phospholipid TFA concentrations across more than 8000 participants of European descent, as part of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium (11). To our knowledge, this is the first report of this genetic association. In addition, we tested whether the top associations we observed from our GWAS meta-analyses in European populations replicated among African Americans, Chinese, and Hispanic populations in the discovery cohorts. We also evaluated the extent to which the associations of single-nucleotide polymorphisms (SNPs) with TFA concentrations that did replicate might be affected by differences in circulating concentrations of *cis*-PUFAs, which may compete with TFA in their metabolism (8).

METHODS

Populations

We performed a prospectively designed, prespecified pooled GWAS analyses including 8013 participants of European descent across 7 cohort studies participating in the CHARGE Consortium Fatty Acids Working Group. The cohorts were the Coronary Artery Risk Development in Young Adults (CARDIA), Cardiovascular Health Study (CHS), Genetics of Lipid-Lowering Drugs and Diet Network (GOLDN), Health Professionals Follow-Up Study (HPFS), Multi-Ethnic Study of Atherosclerosis (MESA), Nurses' Health Study (NHS), and Women's Genome Health Study (WGHS) (**Table 1**). All participants provided informed written consent, including consent to participate in genetic studies and all studies received approval from local ethical oversight committees. Details of participating cohorts are presented in the **Supplemental Methods**.

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[4]Although the cohort studies participating in this study were not clinical trials, several have been registered. The ClinicalTrials.gov identifiers are as follows: CHS, NCT00005133; GOLDN, NCT00083369; MESA, NCT00005487; and WHS (parent study of WGHS), NCT00000479. ISRCTN, HPFS, and NHS have not been registered.

[5]DM, EKK, COJ, and RNL contributed equally to this work.

[6]Supplemental Methods, Supplemental Tables 1–9, and Supplemental Figure 1 are available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[8]Abbreviations used: CARDIA, Coronary Artery Risk Development in Young Adults; CHARGE, Cohorts for Heart and Aging Research in Genomic Epidemiology; CHS, Cardiovascular Health Study; *FADS*, fatty acid desaturase; GOLDN, Genetics of Lipid-Lowering Drugs and Diet Network; GWAS, genome-wide association study; HPFS, Health Professionals Follow-Up Study; MAF, minor allele frequency; MESA, Multi-Ethnic Study of Atherosclerosis; NHS, Nurses' Health Study; SNP, single-nucleotide polymorphism; TFA, *trans* fatty acid; WGHS, Women's Genome Health Study.

TABLE 1
Characteristics of participants of European ancestry in the CHARGE Consortium Fatty Acids Working Group[1]

				Circulating phospholipid fatty acid concentration, % of total fatty acids[2]				
Cohort	*n*	Age, y	Women, %	*trans*-16:1n–7	total *trans*-18:1	*cis*/*trans*-18:2	*trans*/*cis*-18:2	*trans*/*trans*-18:2
CARDIA	1507	45.8 ± 3.4	53.3	0.056 ± 0.028	1.489 ± 0.632	0.048 ± 0.021	0.133 ± 0.053	0.030 ± 0.021
CHS	2404	75.0 ± 5.1	61.6	0.190 ± 0.05	2.010 ± 0.71	0.080 ± 0.02	0.130 ± 0.05	0.050 ± 0.04
GOLDN	793	48.3 ± 15.9	50.4	0.073 ± 0.029	1.442 ± 0.424	0.086 ± 0.023	0.097 ± 0.032	0.043 ± 0.024
HPFS	1295	64.3 ± 8.6	0	0.137 ± 0.045	1.498 ± 0.639	0.116 ± 0.049	0.085 ± 0.043	0.014 ± 0.018
MESA	707	61.6 ± 10.4	61.6	0.064 ± 0.027	1.493 ± 0.675	0.062 ± 0.021	0.135 ± 0.059	0.038 ± 0.023
NHS	655	59.9 ± 6.5	100	0.152 ± 0.045	1.619 ± 0.711	0.176 ± 0.064	0.135 ± 0.055	0.087 ± 0.059
WGHS	652	54.4 ± 6.5	100	0.055 ± 0.026	1.791 ± 0.527	0.089 ± 0.023	0.098 ± 0.048	0.033 ± 0.012

[1]Values are means ± SDs unless otherwise indicated. CARDIA, Coronary Artery Risk Development in Young Adults Study; CHARGE, Cohorts for Heart and Aging Research in Genomic Epidemiology; CHS, Cardiovascular Health Study; GOLDN, Genetics of Lipid-Lowering Drugs and Diet Network; HPFS, Health Professionals Follow-Up Study; MESA, Multi-Ethnic Study of Atherosclerosis; NHS, Nurses' Health Study; WGHS, Women's Genome Health Study.

[2]Fatty acids were measured in erythrocyte membrane phospholipids (GOLDN, HPFS, and NHS) or plasma phospholipids (CARDIA, CHS, MESA, and WGHS).

Measurements of phospholipid fatty acids

Detailed methods of phospholipid fatty acid measurements are provided in the Supplemental Methods. Four cohorts measured erythrocyte phospholipids, and 3 measured plasma phospholipids; phospholipid fatty acid concentrations in these 2 compartments interchange with each other and are reasonably correlated (12, 13), with correlations of 0.68 for *trans*-16:1n–9, 0.71–0.84 for *trans*-18:1 isomers, and 0.56 for *trans*-18:2 (I King, University of New Mexico, personal communication, October 7, 2014). Concentrations of each TFA were expressed as weight percentage of total fatty acids. We separately evaluated *trans*-16:1n–7, total *trans* 18:1 (all available isomers), and *trans*/*cis*, *cis*/*trans*, and *trans*/*trans*-18:2. *Trans*-18:1 isomers were summed given their very high intercorrelations (in each cohort, the correlation between each individual *trans*-18:1 isomer and total *trans*-18:1 was >0.90). In contrast, concentrations of *trans*-16:1n–7, total *trans*-18:1, and the 3 *trans*-18:2 isomers were only modestly intercorrelated (**Supplemental Tables 1–7**), consistent with heterogeneous dietary sources and/or metabolic determinants.

Genotyping and genome-wide analysis

Genotyping was done in each cohort separately by using high-density SNP marker platforms (CARDIA, GOLDN, and MESA: Affymetrix 6.0; HPFS and NHS: Illumina 550k, 610Q, 660Q, Affymetrix 6.0; CHS Caucasians: Illumina 370; CHS African Americans: Illumina Omni 1M; WGHS: Illumina HumanHap300 Duo+). Samples with call rates <95%–98% (depending on the cohort) at genotyped markers were excluded. Genotypes were imputed to approximately 2.5 million HapMap SNPs by using Beagle (14) (CARDIA), Bimbam (15) (for Caucasians) and Beagle (14) (for African Americans) (CHS), Impute (16) (MESA), or Mach (17) (GOLDN, HPFS, NHS, and WGHS). SNPs for which Hardy-Weinberg equilibrium testing resulted in $P < 10^{-4}$ to $< 10^{-6}$ (cohort-specific) were excluded from imputation. Additional details on genotyping and imputation in each cohort are provided in the Supplemental Methods.

Association analysis between genotype and each fatty acid was performed separately within each cohort according to a prespecified analysis plan. All studies conducted linear regression analysis with robust variance estimators by using an additive genetic model (i.e., regression of phenotype on the number of reference alleles) or equivalently the imputed dosage for imputed genotypes. All analyses adjusted for age, sex, site of recruitment where appropriate, and population substructure by using principal component analysis [derived in each cohort from subsets (e.g., 100,000–200,000) of the genotyped SNPs] to account for possible population genetic substructure. We did not consider potential effects of pharmacotherapy on gene-TFA associations, given no known effects of common medications on TFA concentrations.

Meta-analysis

For each SNP and fatty acid, study-specific GWAS results were combined by using inverse-variance weighted meta-analysis (Metal, www.sph.umich.edu/csg/abecasis/metal). SNPs with minor allele frequency (MAF) ≤1% and imputation quality <0.30 were excluded from the meta-analyses, with visual evaluation of Q-Q plots to assess the potential for systematic bias. *P* values $<5 \times 10^{-8}$ were considered statistically significant in the meta-analysis of GWASs among Caucasians. The proportion of variance in fatty acid concentrations explained by a particular variant allele was calculated from the formula $[\beta^2 \times 2 \times \text{MAF}(1 - \text{MAF})]/\text{Var}(Y)]$, where β was the regression coefficient for one copy of the allele; MAF, the minor allele frequency; and Var(Y), the variance of the fatty acid.

Analyses of top SNPs in cohorts of African, Chinese, and Hispanic descent

We investigated whether genome-wide significant SNPs identified in the meta-analysis were associated with phospholipid TFA concentrations in African American (CHS and MESA; n = 1082), Chinese American (MESA; n = 669), and Hispanic American (MESA; n = 657) populations. The SNPs were directly genotyped as part of candidate gene studies in MESA or were available from genome-wide scans in the CHS. We used linear regression and additive models as described above. We used Bonferroni correction to adjust for the number of genome-wide significant SNPs evaluated in these minority populations (α = 0.05/number of SNPs), with the association in each

minority population considered a separate hypothesis. In addition, because our top hit with 18:2ct replicated in Chinese and Hispanics but not in African Americans, we explored the full loci of 2 genes in this region [fatty acid desaturase (*FADS*) 1/2 region: 61566604–61635303, ~70 kb, in Human Genome build 37; 61323180–61391879 in build 36] in African Americans by using fine mapping (1000 Genomes imputation data) to assess whether absence of top hit association might be due to differences in genetic architecture between races. We used Bonferroni correction to adjust for the 123 independent SNPs in this fine mapping region, estimated by using an eigendecomposition approach (18, 19) ($\alpha = 0.05/123 = 0.004$).

Influence of other fatty acids

Because one of the identified associations was in a gene strongly associated with circulating concentrations of *cis*-PUFA (20), which may compete with the metabolism of TFA by means of substrate inhibition, product inhibition, or competitive inhibition (8), we also evaluated the extent to which the observed significant associations between the top SNP in this region and TFA concentrations might change with differences in circulating concentrations of *cis*-PUFA. We also evaluated potential mediation of the association by total *trans*-18:1. Each significant SNP-TFA association, previously adjusted for age, sex, study site, and population substructure, was further evaluated in each cohort with and without further adjustment for specific individual circulating fatty acids, including total *trans*-18:1, individual *cis* n–3 PUFA (18:3n–3, α-linolenic acid; 20:5n–3, eicosapentaenoic acid; 22:5n–3, docosapentaenoic acid; 22:6n–3, docosahexaenoic acid), and individual *cis* n–6 PUFA (18:2n–6, linoleic acid; 20:4n–6, arachidonic acid; 22:4n–6, adrenic acid). We qualitatively compared the proportional difference (ratio of β-coefficients) as well as the 95% CI in the pooled SNP-TFA association with and without adjustment for each *cis* fatty acid.

RESULTS

The characteristics of participants in the cohorts included in the meta-analyses are described in Table 1 as well as in prior reports (20, 21). Briefly, the mean (±SD) age in the participating cohorts ranged from 45.8 ± 3.4 to 75.0 ± 5.1 y. Five TFAs measured in the phospholipid fraction were available for analysis: *trans*-16:1n–7, total *trans*-18:1, *cis*/*trans*-18:2, *trans*/*cis*-18:2, and *trans*/*trans*-18:2. As expected, total *trans*-18:1 was the most abundant TFA, with mean concentration as percentage of total fatty acids ranging from 1.44 ± 0.42 in GOLDN to 1.79 ± 0.53 in WGHS.

Genome-wide associations with circulating TFAs

In meta-analyses of the 7 European populations, no significant associations were identified for *trans*-16:1n–7, *trans*-18:1, or *trans*/*cis*-18:2 (**Figure 1, Supplemental Table 8**). A total of 31 SNPs on chromosome 11 attained genome-wide significance for *cis*/*trans*-18:2. These SNPs were in moderate to high linkage disequilibrium with each other and located in or near the *FADS1/2* gene cluster; a top SNP was rs174548 ($P = 4.90 \times 10^{-15}$) (**Figure 2, Table 2**). Each copy of the G allele of rs174548 was associated with 0.0035 unit (percentage of total fatty acids) higher concentrations of *cis*/*trans*-18:2, with consistent direction of association across all 7 cohorts. Across cohorts, the single variant explained an average of 1.1% of the variation in circulating *cis*/*trans*-18:2.

The T allele in SNP rs10469266 on chromosome 18 was nominally associated with higher concentrations of *trans*/*trans*-18:2 ($P = 2.34 \times 10^{-8}$). However, this SNP had a very low MAF (1.6%) and was not located in any known gene, and its nominal association was largely driven by association within a single cohort (CARDIA), with missing data in 2 cohorts (Supplemental Table 2). We therefore did not further evaluate this likely spurious finding.

Associations in participants of African, Chinese, and Hispanic descent

We next evaluated the associations of rs174548 with *cis*/*trans*-18:2 concentrations in non-European participants from these cohorts (Bonferroni-corrected $\alpha = 0.05$). This association replicated in Hispanic Americans ($P = 1.05 \times 10^{-6}$) and Chinese Americans ($P = 0.002$) but not in African Americans ($P = 0.34$) (Table 2). Because of differences in MAFs for rs174548 on chromosome 11 between races, we used fine mapping to further evaluate this *FADS1* and *FADS2* region for association with *cis*/*trans*-18:2 in African Americans. The meta-analysis demonstrated a top hit of rs174579 in the *FADS2* region ($P = 4.05 \times 10^{-5}$), meeting Bonferroni-corrected statistical significance ($\alpha = 0.004$) (**Supplemental Figure 1**).

Among European-ancestry participants, 8 other SNPs outside the *FADS1/2* region approached genome-wide significance—namely, rs16958148 ($P = 8.49 \times 10^{-8}$), rs16894446 ($P = 2.74 \times 10^{-7}$), rs17099388 ($P = 3.63 \times 10^{-7}$), rs11104877 ($P = 3.83 \times 10^{-7}$), rs7566684 ($P = 8.39 \times 10^{-7}$), rs1399212 ($P = 3.07 \times 10^{-7}$), rs11248534 ($P = 4.99 \times 10^{-7}$), and rs5752209 ($P = 2.94 \times 10^{-7}$) (Supplemental Table 8). In exploratory analyses, none of these 8 SNPs was significantly associated with TFA concentrations in any of the non-European populations ($P > 0.05 \div 8 = 0.00625$ each) (**Supplemental Table 9**).

Influence of other fatty acids

The association of rs174548 in *FADS1/2* with *cis*/*trans*-18:2 remained statistically significant after adjusting for circulating concentrations of *trans*-18:1 and various individual n–3 and n–6 PUFAs except for *cis*-20:4n–6 (arachidonic acid) (**Table 3**). After adjustment for 20:4n–6, the association between rs174548 and *cis*/*trans*-18:2 was nearly completely attenuated and no longer statistically significant (β-coefficient: 0.0005; 95% CI: −0.00048, 0.00148; $P = 0.33$). Similar findings were seen among Chinese American and Hispanic American populations in MESA, in whom the association between rs174548 and *cis*/*trans*-18:2 was no longer evident after further adjustment for phospholipid concentrations of 20:4n–6 (data not shown). Interestingly, the correlation between phospholipid 20:4n–6 and *cis*/*trans*-18:2 concentrations was not high ($r = -0.19$). In analyses within the CHS cohort in which 20:4n–6 was the dependent variable, rs174548 remained significantly inversely associated with 20:4n–6 concentrations after adjustment for *cis*/*trans* 18:2 concentrations (β-coefficient: −1.74; $P = 5 \times 10^{-253}$).

DISCUSSION

This meta-analysis of 7 separate cohorts provides novel evidence that circulating concentrations of certain phospholipid

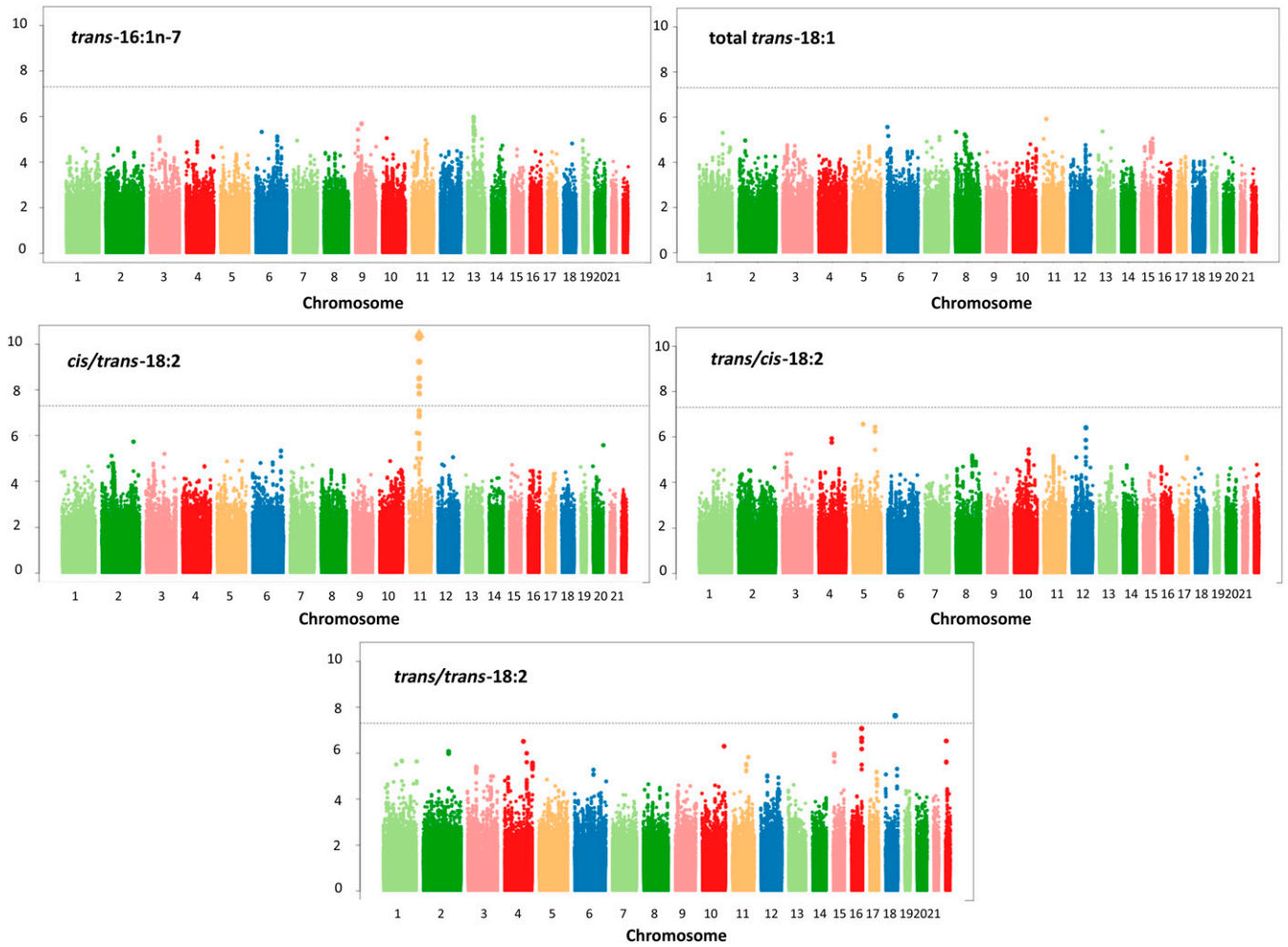

FIGURE 1 Meta-analyses of genome-wide associations for circulating concentrations of *trans* fatty acids.

TFAs are under genetic control. Specifically, associations of multiple SNPs on chromosome 11 suggested that concentrations of *cis*/*trans*-18:2 are influenced by common genetic variation located in or near the *FADS1/2* gene cluster. Findings were consistent in populations of European, Chinese, and Hispanic ancestry, and hits in the *FADS2* region were also identified by using fine mapping among African Americans, suggesting that this genetic control is conserved across several races. In contrast, no evidence for significant genetic control was identified for other TFAs, including *trans*-16:1n–7, *trans*-18:1, *trans*/*cis*-18:2, and *trans*/*trans*-18:2.

The *FADS1/2* cluster encodes fatty acid desaturase enzymes that regulate endogenous metabolism of *cis* n–3 and n–6 PUFA (20, 22). The association of this important regulatory region with circulating concentrations of *cis*/*trans*-18:2 implies, for the first time to our knowledge, that this region also plays a role in the metabolism of certain TFAs. Our findings further suggest that this association was mediated by circulating concentrations of arachidonic acid, the archetypical n–6 *cis*-PUFA product of linoleic acid, in that adjustment for circulating concentrations of arachidonic acid largely abolished the association of the *FADS1/2* region with *cis*/*trans*-18:2. Arachidonic acid, a key modulator of inflammatory pathways and cellular signaling, is a precursor to eicosanoids that regulate production of leukotrienes, prostaglandins, prostacyclin, and thromboxanes and also a precursor to active resolvers of inflammation such as lipoxins and epoxyeicosatrienoic acids (23, 24). Our demonstration of a relation between variation in *FADS1/2*, *cis*/*trans*-18:2, and arachidonic acid suggests novel pathways of shared influences by *cis*- and *trans*-PUFA. For example, the effect of genetic variation in the *FADS1/2* gene cluster on *cis*/*trans*-18:2 could be mediated by effects of the variant on arachidonic acid. We have previously shown that genetic variation in the *FADS1/2* cluster explains more than 20% of variation in circulating arachidonic concentrations, with suggestions that the G allele of rs174548 may reflect lower *FADS1/2* activity, resulting in lower conversion of linoleic to arachidonic acid (25). Subsequent differences in arachidonic acid concentrations could then alter activities of FADS2 and stearoyl-CoA desaturase, enzymes that could be involved in metabolism (or, less likely, synthesis) of *cis*/*trans*-18:2. Arachidonic acid could also alter the rate of incorporation or removal of *cis*/*trans*-18:2 from phospholipids, for instance, by influencing concentrations or activities of lysophosphatidyl acyltransferase or phospholipase A2, respectively. The *FADS1/2* cluster could also be directly involved in the metabolism of *cis*/*trans*-18:2, for example, converting this fatty acid to (unmeasured) *trans*-arachidonic acid isomers. In this case, the attenuation of this association following adjustment for arachidonic acid could reflect the correlation of this same genetic variant with arachidonic acid,

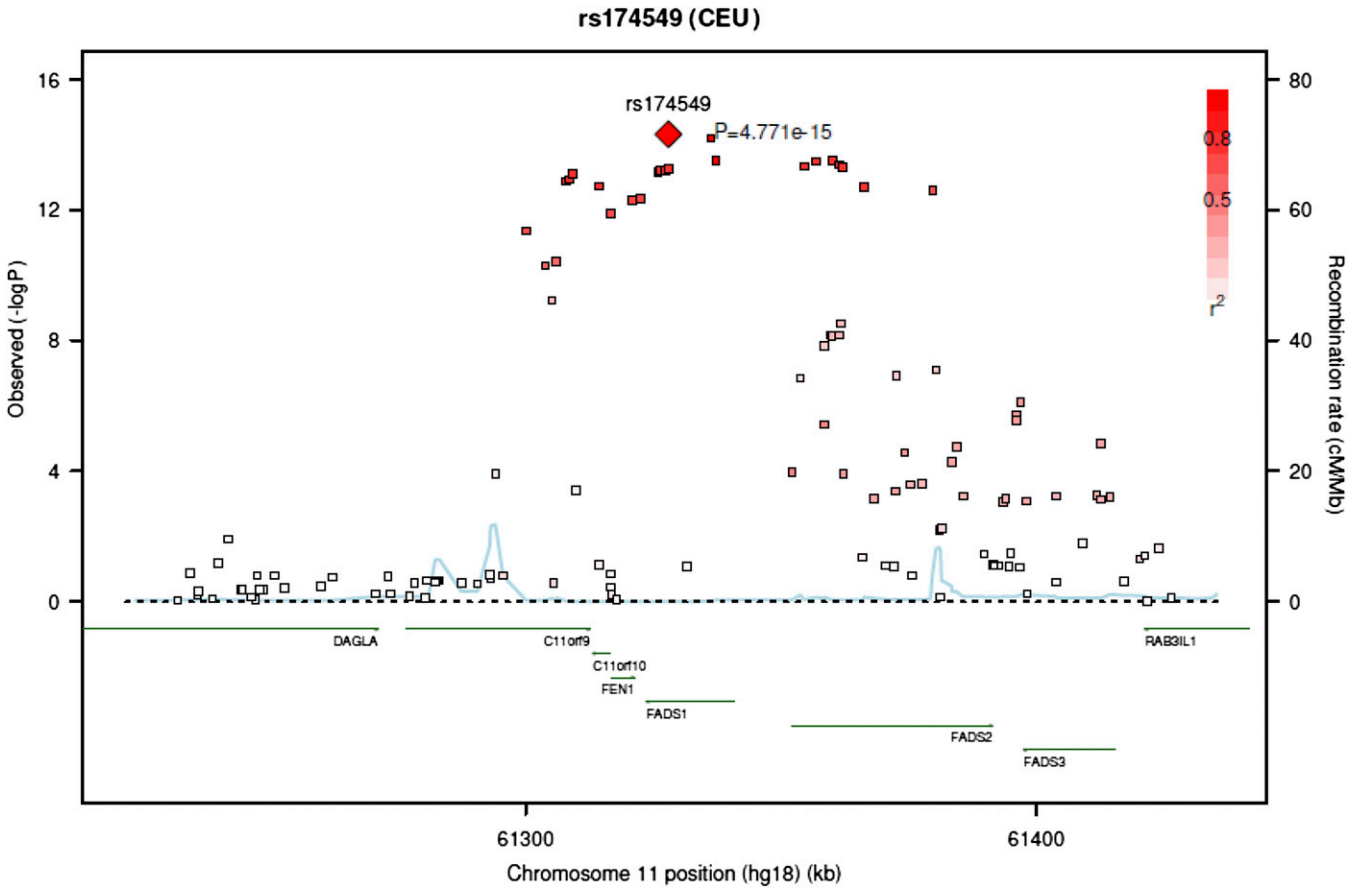

FIGURE 2 Regional association plot for SNP rs174549 and *cis*/*trans*-18:2, showing high linkage disequilibrium with 30 other significantly associated SNPs in an area of genes centered on the *FADS1/2* cluster. rs174549 was in strong linkage disequilibrium ($r^2 = 0.95$) with rs174548 ($P = 4.90 \times 10^{-15}$), on which we focus in this article for comparability with our prior analysis of circulating n–3 fatty acid concentrations (20). *FADS*, fatty acid desaturase; SNP, single-nucleotide polymorphism.

the concentrations of which may be a sensitive indicator of the flow into the pathway and the genetic variation in *FADS1/2*. Our novel findings support the need for further investigation of the molecular pathways underlying the relationships between these iconic fatty acids and genetic regions, providing insights into novel regulatory pathways of *cis* and *trans* fatty acids.

Genetic variation in the *FADS1/2* gene cluster is linked to other traits, including blood lipids (total cholesterol, LDL, HDL, and triglycerides), glycemic responses (glucose and β-cell function), and even resting heart rate (26–31). Relationships of the *FADS1/2* region with clinical coronary events have been conflicting, with some but not all studies demonstrating associations (32–38). The specific functional variants and mechanistic pathways underlying these associations remain unclear. Our newly identified association with circulating *cis*/*trans*-18:2, as well as its interrelationship with arachidonic acid, provides additional impetus to elucidate the functional significance of the identified genetic variants and their role in modulating lipid and metabolic traits.

Although we found variation in the *FADS1/2* locus to be related to *cis*/*trans*-18:2 concentrations in whites, Chinese, Hispanics, and blacks, the identified variants were not the same in blacks. Recent analyses suggest that whites and blacks have functional differences in *FADS1/2* genetic variants related to PUFA metabolism, perhaps driven by evolutionary adaption to facilitate migration from coastal to inland regions on the African continent (39–41). Nonetheless, although the specific evolutionary history and overall functional activity of this gene cluster may differ between whites and blacks, our findings suggest the presence of a conserved general influence of the *FADS1/2* locus on *cis*/*trans*-18:2 concentrations.

We did not observe significant genome-wide associations with circulating concentrations of other TFAs. Prior investigations from this CHARGE fatty acid consortium demonstrate ample statistical power to detect associations of genetic variants with other fatty acids, including *cis* n–3 PUFA and saturated and monounsaturated fatty acid products of de novo lipogenesis (20, 21). These findings together suggest that circulating concentrations of most specific TFAs are not subject to appreciable genetic control; this is consistent with the dietary source of these TFAs and the inability of mammals to synthesize *trans* double bonds. These findings also imply that prior observations of relationships between circulating concentrations of these TFAs with risk factors and disease outcomes (3–6) might not be substantially influenced by endogenous metabolic processes and support the use of these measurements as biomarkers of dietary exposure.

Our investigation has several strengths. Phospholipid fatty acid measurements represent objective circulating biomarkers that also reflect the biologically relevant pool of membrane and tissue

TABLE 2
Top SNPs significantly associated with phospholipid *cis*/*trans*-18:2 concentrations[1]

Population	*n*	Chromosome	Genes of interest	SNP	Coded allele (frequency)	*P* value	β-coefficient ± SE
Overall GWAS meta-analysis							
European ancestry	8103	11	*FADS1/2*	rs174549[2]	A/G (0.29)	4.77×10^{-15}*	0.0035 ± 0.0004
				rs174548[2]	G/C (0.29)	4.90×10^{-15}*	0.0035 ± 0.0004
Replication in non-European ancestry populations							
Hispanic Americans	657	11	*FADS1/2*	rs174548	G/C (0.53)	1.05×10^{-6}**	0.0053 ± 0.0011
Chinese Americans	669				G/C (0.58)	0.002**	0.0028 ± 0.0009
African Americans	1082				G/C (0.21)	0.34**	0.0009 ± 0.0010
Fine mapping of *FADS1/2* region in African Americans							
African Americans	1082	11	*FADS1/2*	rs174579	T/C (0.05)	4.05×10^{-5}***	0.0118 ± 0.0029

[1]*Evaluated at genome-wide significance ($\alpha = 5.0 \times 10^{-8}$); **a single SNP (rs174548) was evaluated at $\alpha = 0.05$ for each race-ethnicity; ***evaluated with Bonferroni correction for the number of independent SNPs in this region ($\alpha = 0.05/123 = 0.004$). *FADS*, fatty acid desaturase; GWAS, genome-wide association study; SNP, single-nucleotide polymorphism.

[2]A total of 29 other SNPs in this region, in moderate to high linkage disequilibrium with rs174549 and rs174548, were also significantly associated with *cis*/*trans*-18:2 concentrations in the meta-analysis, with *P* values ranging from 6.34×10^{-15} to 1.45×10^{-8} (see Supplemental Table 3). rs174549, which showed the strongest association with *cis*/*trans*-18:2 concentrations, was in strong linkage disequilibrium ($r^2 = 0.95$) with rs174548. We focus on rs174548 in this article for comparability with our prior analysis of circulating n–3 fatty acid concentrations (20).

phospholipids. By evaluating genetic predictors of circulating TFAs across 7 cohorts and integrating these findings through meta-analysis, we increased statistical power as well as generalizability. Findings observed in participants of European ancestry were evaluated in Chinese, Hispanic, and African American populations, including using fine mapping in the African Americans. We evaluated potential interrelationships of circulating *cis*-PUFAs with the observed genetic associations, providing novel evidence for a possible biologic interaction between arachidonic acid, *cis*/*trans*-18:2, and the *FADS1* locus.

Potential limitations should be considered. We investigated genetic associations, and the biological effects of the identified variants on circulating concentrations of these fatty acids are unknown. The top SNPs that were identified from the GWAS of *cis*/*trans*-18:2 are in high linkage disequilibrium with many SNPs in the region, and resequencing of the region will be needed to identify the causal variant(s). As with all genetic association studies of complex traits, the proportion of variance explained was small. The TFAs were measured in plasma phospholipids in some cohorts and erythrocyte phospholipids in others. Yet, although mean concentrations of specific fatty acids can differ in these 2 compartments, plasma and membrane phospholipids freely interexchange, their concentrations are well correlated (12, 42), and the direction of association for the top SNPs was consistent in all 7 cohorts. The study included relatively small numbers of non-Caucasians; nonetheless, we confirmed association of the top SNP or another in the same region in each non-Caucasian ethnic group.

In summary, we identified novel evidence for genetic control of circulating phospholipid concentrations of *cis*/*trans*-18:2 by the *FADS1/2* cluster, and that such regulation may be mediated by concentrations of arachidonic acid, an n–6 *cis*-PUFA.

TABLE 3
Meta-analyses for the relation between rs174548 in the *FADS1* gene and *cis*/*trans*-18:2 concentrations before and after adjusting for other individual circulating fatty acids also measured in erythrocyte membrane or plasma phospholipids[1]

Further adjustment for phospholipid fatty acid concentrations	Association between rs174548 and *cis*/*trans*-18:2[2]	*P* value
None	0.0035 ± 0.0004	4.9×10^{-15}
Total *trans*-18:1	0.0033 ± 0.0004	1.12×10^{-19}
18:3n–3 (α-linolenic acid)	0.0031 ± 0.0005	4.29×10^{-12}
20:5n–3 (eicosapentaenoic acid)	0.0027 ± 0.0004	5.89×10^{-10}
22:5n–3 (docosapentaenoic acid)	0.0029 ± 0.0004	9.02×10^{-11}
22:6n–3 (docosahexaenoic acid)	0.0031 ± 0.0004	1.48×10^{-12}
18:2n–6 (linoleic acid)	0.0022 ± 0.0005	1.28×10^{-6}
20:4n–6 (arachidonic acid)	0.0005 ± 0.0005	0.33
22:4n–6 (adrenic acid)	0.0049 ± 0.0008	2.23×10^{-10}

[1]Findings presented were among Caucasians. Results were similar in the non-European populations: the association among Chinese Americans was no longer significant after adjusting for either linoleic acid or arachidonic acid, the association among Hispanic Americans was no longer significant after adjusting for arachidonic acid, and further adjustment for the other fatty acids in this table did not appreciably alter the association between rs174548 and *cis*/*trans*-18:2 among Chinese or Hispanic Americans. *FADS*, fatty acid desaturase.

[2]Values are β-coefficients ± SEs.

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The authors' responsibilities were as follows—DM, EKK, DKA, SSR, EBR, FBH, DIC, and M Fornage: designed the research (project conception, development of overall research plan, study oversight); DM, EKK, MRI, DKA, MYT, SSR, QS, MKJ, EBR, FBH, LW, PMR, DIC, M Fornage, LS, IBK, BMP, DSS, and IY-DC: conducted the research (hands-on conduct of the experiments and data collection); DM, DKA, MYT, SSR, QS, MKJ, LW, PMR, DIC, M Fornage, and LS: provided essential reagents or provided essential materials; EKK, COJ, RNL, H Wiener, MRI, AM, QS, H Wu, LW, DIC, AYC, M Foy, and BM: analyzed data or performed statistical analysis; DM, EKK, RNL, LD, and JHYW: wrote the manuscript; and DM, EKK, and QS: had primary responsibility for the final content. DM reports ad hoc honoraria from Bunge and membership on the Unilever North America Scientific Advisory Board. Harvard University has filed a provisional patent application, which has been assigned to Harvard University, listing DM as a coinventor to the US Patent and Trademark Office for use of *trans*–palmitoleic acid to prevent and treat insulin resistance, type 2 diabetes, and related conditions. BMP serves on the Data Safety Monitoring Board for a clinical trial funded by the device manufacturer (Zoll LifeCor) and on the Steering Committee of the Yale Open Data Access Project funded by Johnson & Johnson. The other authors reported no conflicts of interest.

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