Online Supplemental Materials

Genetic Loci Associated with Circulating Phospholipid Trans Fatty Acids - A Meta-Analysis of Genome-Wide Association Studies from the CHARGE Consortium

Details of Participating Cohorts:

Study Samples

Participants for the current analysis were drawn from 7 cohort studies, including the Coronary Artery Risk Development in Young Adults Study (CARDIA), the Cardiovascular Health Study (CHS), the Genetics of Lipid-Lowering Drugs and Diet Network (GOLDN), the Health Professionals Follow-up Study (HPFS), the Multi-Ethnic Study of Atherosclerosis (MESA), the Nurses Health Study (NHS), and the Women's Genome Health Study (WGHS), All participants provided informed consent. Local ethical committees at each institution approved the individual study protocols.

The Coronary Artery Risk Development in Young Adults Study (CARDIA)

The CARDIA Study is a prospective multicenter study with 5115 adults Caucasian and African American participants of the age group 18-30 years, recruited from four centers. The recruitment was done from the total community in Birmingham, AL, from selected census tracts in Chicago, IL and Minneapolis, MN; and from the Kaiser Permanente health plan membership in Oakland, CA. The details of the study design for the CARDIA study have been published before (1). Eight examinations have been completed since initiation of the study in 1985–1986, respectively in the years 0, 2, 5, 7, 10, 15, 20 and 25. Written informed consent was obtained from participants at each examination and all study protocols were approved by the institutional review boards of the participating institutions.

CARDIA Study samples from were genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0 (Santa Clara, California); only participants of European descent were included in the GWAS analyses. Genotyping was completed for 1720 individuals with a sample call rate $\geq$ 98%. A total of 578,568 SNPs passed quality control (MAF $\geq$ 2%, call rate $\geq$ 95%, HWE $\geq 10^{-4}$) and were used for imputation. For this study, complete genotype and phenotype information were available for 1507 individuals.

The fatty acids were measured in stored (–70°C) EDTA plasma, drawn in the fasting state, using previously described methods.(2) Lipids are extracted from the plasma using a chloroform/methanol extraction method and the cholesterol esters, triglyceride, phospholipids and free fatty acids are separated by thin layer chromatography. The fatty acid methyl esters are obtained from the phospholipids and are detected by gas chromatography flame ionization. The concentration of each individual fatty acid (28 total identified) was expressed as a percentage of total area under the peaks.

The Cardiovascular Health Study (CHS)

The CHS is a population-based longitudinal study of risk factors for cardiovascular disease and stroke in adults 65 years of age or older, recruited at four field centers (Forsyth County, NC; Sacramento County, CA; Washington County, MD; Pittsburgh, PA) (3). Overall, 5201 predominantly Caucasian individuals were recruited in 1989-1990 from random samples of Medicare eligibility lists, followed by an additional 687 African-Americans recruited in 1992-1993 (total n=5,888). The CHS genome-wide association study (GWAS) in participants of European ancestry, which had the primary aim of studying incident cardiovascular events, focused on 3980 CHS participants who were free of clinical cardiovascular disease at study baseline, consented to genetic testing, and had DNA available for genotyping. A total of 1,908 persons of European ancestry were excluded from the GWAS study sample due to the presence at study baseline of coronary heart disease, congestive heart failure, peripheral vascular disease, valvular heart disease, stroke, or transient ischemic attack.

CHS Study samples from participants of European ancestry were genotyped using the Illumina HumanCNV370-Duo BeadChip system. Samples from participants of African ancestry were genotyped using the Illumina HumanOmni 1-Quad_v1 BeadChip system. Genotyping was successful in 3,291 European American subjects and 823 African American subjects. Participants were eligible for the present investigation if their genotyping was complete and they had available phenotype information. Samples with call rate <95% were excluded. A total of 306,655 autosomal SNPs (European American samples) and 940,567 autosomal SNPs (African American samples) were used in imputation after filtering out SNPs with HWE deviation $p \leq 1 \times 10^{-5}$, call rate ≤97%, zero heterozygote frequency, missing from dbSNP, and >1 duplicate error or Mendelian inconsistency.

Fatty acids were measured on blood samples collected in the 3rd year of followup, drawn after a 12-hour fast and stored at -70^{o}C. Fatty acid measurements were performed at the Fred Hutchinson Cancer Research Center, providing quantitative measurement of 42 fatty acids. Total lipids were extracted from plasma using methods of Folch, and phospholipids separated from neutral lipids by one-dimensional TLC. Fatty-acid-methyl-ester (FAME) samples were prepared by direct transesterification using methods of Lepage and separated using gas chromatography (Agilent5890 gas-chromatograph-FID-detector; Supelco fused-silica 100m capillary column SP-2560; initial 160°C 16 min, ramp 3.0°C/min to 240°C, hold 15 min). Identification, precision, and accuracy were continuously evaluated using model mixtures of known FAMEs and established in-house controls, with identification confirmed by GC-MS at USDA (Peoria, IL). Laboratory CVs were 2.6% for t-16:1n-7, 1.7% for total t-18:1, 7.8% for c/t-18:2, 4.0% for t/c-18:2, and 8.2% for t/t-18:2. The concentration of each individual fatty acid was expressed as a percentage of total area under the peaks.

Genetics of Lipid-Lowering Drugs and Diet Network (GOLDN) family study

The GOLDN study enrolled 1,328 Caucasian American men and women from Minneapolis, MN and Salt Lake City, UT.(4, 5) The main aim was to evaluate the genetic basis for variable response of serum triglycerides to two environmental interventions, one that increased serum triglycerides (acute dietary fat in the form of a milkshake), and one that reduced serum triglycerides (fenofibrate therapy for 3 weeks). Prior to baseline blood sampling, drawn in the fasting state, participants were asked to suspend their lipid medications, and a blood sample for DNA extraction and biochemical measurements was drawn.

Detailed procedures for DNA extraction, purification, genotyping and imputation are described elsewhere.(5) Briefly, 906,600 SNPs were genotyped using the Affymetrix Genome-Wide Human 6.0 array; those that were monomorphic (55,530) or had a call rate <96% (82,462) were excluded from the analysis. Other SNPs were excluded according to the number of families with Mendelian errors, departure from Hardy-Weinberg equilibrium ($P < 10^{-6}$), MAF<1%, missing strand information, or discrepancies with the *mlinfo* file. The remaining SNPs were used in MACH software (Version 1.0.16) to impute the ungenotyped SNPs based on Human Genome Build 36 as the reference. A hybrid dataset from 793 GOLDN participants and with a total of 2,543,887 SNPs, of which 584,029 were initially genotyped, was created and used for the current analyses.

Fatty acids in erythrocyte membranes were extracted with a mixture of chloroform:methanol (2:1, by volume), collected in heptane, and injected onto a capillary Varian CP7420 100m column using a Hewlett Packard 5890 gas chromatograph equipped with a HP6890A autosampler.(6) The initial temperature of 190 ºC was increased to 240 ºC over 50 minutes to separate fatty acids from 12:0 through 24:1n9. The concentration of each individual fatty acid was expressed as a percentage of total area under the peaks.

The Health Professionals Follow-up Study (HPFS) and Nurses' Health Study (NHS)

The HPFS enrolled 51,529 US health professionals (dentists, optometrists, pharmacists, podiatrists, and veterinarians), aged 40 to 75 years, from all 50 states who completed a detailed baseline questionnaire that included questions on medical history, lifestyle, and a comprehensive diet survey in 1986. The NHS enrolled 121,700 registered female nurses, age 30 to 55 years, who lived in one of 11 states and completed a similar detailed baseline questionnaire in 1976. In both cohorts, follow-up questionnaires have been administered biennially to update information on exposures and newly diagnosed diseases. In 1980, 1984, 1986 and every 4 years thereafter, a validated food frequency questionnaire has been sent to NHS participants to collect and update information on diet, alcohol, and

vitamin supplements. In the HPFS the same questionnaire has been sent to HPFS participants every 4 years. In 1993-1995, blood samples were obtained from 18,224 HPFS participants, and in 1989-1990, blood samples were collected from 32,826 NHS participants; fasting status was not required. Upon arrival whole blood samples were centrifuged and stored in cryotubes as plasma, buffy coat, and red blood cells in the vapour phase of liquid nitrogen freezers (–70°C or less). DNA was extracted from the buffy coat fraction of centrifuged blood with the QLAmp Blood Kit (Qiagen, Chatsworth, California).

A nested case-control study of coronary heart disease (CHD; including non-fatal myocardial infarction and fatal CHD events) was conducted among participants who provided blood samples in both cohorts. Self-reported incidence of CHD events was confirmed by exposure-blinded study physicians through reviewing medical records. Participants with prevalent cardiovascular disease or cancer at blood draw were excluded from the case-control study. Controls were selected randomly using risk-set sampling and matched to cases in a 2:1 ratio on age, smoking, and month of blood return; in the NHS fasting status was further matched. Genotyping was performed using the Affymetrix SNP 6.0 array and the Birdseed calling algorithm. A total of 1354 HPFS samples (98%) and 1153 NHS samples (96%) passed laboratory technical quality control criteria. Population substructure was determined using principal component analysis. A set of 12,021 SNPs with very low levels of linkage disequilibrium and minor allele frequency >0.05 in Caucasians were selected and used to construct the principal components of ethnicity (7). Study participants passing quality control were analyzed together with a set of 209 HapMap II founders (59 CEU, 60 YRI, 45 JPT and 45 CHB). Participants within the means of the first and second principal components [mean (SD) = 3] among self-described Caucasians were classified as having primarily European ancestry. We excluded participants with substantial evidence of non-European genetic ancestry from subsequent analysis. In addition, SNPs that were monomorphic, had a missing call rate ≥2%, a Hardy-Weinberg Equilibrium p-value $<1\times10^{-4}$, or a minor allele frequency <0.02 were excluded, leaving a total of 724,881 SNPs in the HPFS or 721,316 in the NHS that passed quality control analysis of called genotypes. Imputation of ~2.5 million SNPs was performed using MACH software (v1.0.16) with HapMap CEU phased II data (Release 22) as the reference panel.

Erythrocyte fatty acid concentrations were determined by gas-liquid chromatography (8) among 1334 CHD cases and controls in the HPFS and 1131 case-control triplets in the NHS, among whom 1295 subjects in HPFS and 655 in NHS also had genotyping data. Briefly, fatty acids in erythrocytes were first extracted into isopropanol and hexane and then transmethylated with methanol and sulfuric acid. Fatty acid methyl esters were evaporated and redissolved in isooctane and then measured by gas-liquid chromatography. Individual peaks were identified by comparison with known standards, and each peak was quantified by calculating the area under the peak. Laboratory CVs were 22.6% for t-16:1n-7, 27.5% for total t-18:1, 13.7% for c/t-18:2, 16.2% for t/c-18:2, and 53.7% for t/t-18:2 in HPFS; and 13.7% for t-

16:1n-7, 16.6% for total t-18:1, 18.1% for c/t-18:2, 22.6% for t/c-18:2, and 35.5% for t/t-18:2 in NHS. The concentration of each individual fatty acid was expressed as a percentage of total area under the peaks.

The Multi-Ethnic Study of Atherosclerosis (MESA)

The MESA Study is a study of the characteristics of subclinical cardiovascular disease (disease detected non-invasively before it has produced clinical signs and symptoms) and the risk factors that predict progression to clinically overt cardiovascular disease or progression of the subclinical disease.(9) MESA researchers study a diverse, population-based sample of 6,814 asymptomatic men and women aged 45-84. Thirty-eight percent of the recruited participants are white, 28 percent African-American, 22 percent Hispanic, and 12 percent Asian, predominantly of Chinese descent, as well as 2,128 additional individuals from 594 families recruited through MESA Family by utilizing the existing MESA framework, yielding 3,026 sibpairs divided between African Americans and Hispanic-Americans. Participants were recruited from six field centers across the United States: Wake Forest University, Columbia University, Johns Hopkins University, University of Minnesota, Northwestern University and University of California - Los Angeles.

MESA and MESA Family samples were genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0 (Santa Clara, California); for the current meta-analysis only self-reported Caucasian participants were analyzed, while MESA Chinese, African American and Hispanic samples are included in the look-up of top SNPs. Sample exclusion criteria included heterozygosity > 53% and individual-level genotyping callrate < 95%. Monomorphic SNPs were removed, and there was no filter on HWE or MAF. IMPUTE version 2.1.0 was used to perform imputation for the MESA SHARe Caucasian participants (chromosomes 1-22) using HapMap Phase I and II - CEU as the reference panel (release #24 - NCBI Build 36 (dbSNP b126)). Relationship inference was performed using KING(10) to identify first- and second- degree relatives, and an unrelated set of individuals was identified for genome-wide association analysis.

Blood was drawn in the fasting state at the baseline study visit and stored at –70°C . Fatty acids were obtained for a subset of 2,767 individuals with genotypes available through MESA SHARe, with approximately equal representation from the four ethnic groups (713 Caucasians, 712 Chinese, 645 African Americans, and 697 Hispanics). The fatty acids were measured in stored (–70°C) EDTA plasma using previously described methods.(2) Lipids were extracted from the plasma using a chloroform/methanol extraction method, and the cholesterol esters, triglyceride, phospholipids and free fatty acids were separated by thin layer chromatography. The fatty acid methyl esters were obtained from the phospholipids and detected by gas chromatography flame ionization. Laboratory CVs for trans fatty

acids ranged from 10.9% for t-16:1n-7 to 63.5% for c/t-18:2. The concentration of each individual fatty acid (28 total identified) was expressed as a percentage of total area under the peaks.

The Women's Genome Health Study (WGHS)

The WGHS enrolled US women from the ongoing Women's Health Study (WHS) that began in 1992.(11). The women in WGHS (n=23,294) were of self-reported European ancestry, age 45 years or older, and generally healthy at baseline. They have been followed for major incident health events such as myocardial infarction, stroke, venous-thromboembolism, diabetes, cancer, osteoporosis, cognitive decline, and vision disorders. Dietary, behavioral, and medical data are obtained annually. Women having both genotype data and erythrocyte fatty acid profiles (n=652) were included in the current study.

Genomic DNA was extracted from buffy-coats using The MagNA Pure LC System (Roche Molecular Biochemicals). Genotyping was done using Illumina Infinium II assay to call 315,176 haplotype-tagging SNPs (the Human HAP300 panel). Genotype calls were made using Illumina BeadStudio v3.1 software. All SNPs with MAF <1% or HWE $P \leq 10-6$ were excluded.

Blood was drawn (with about 2/3 of particiapants in the fasting state) at the baseline study visit and stored at –70°C. Erythrocyte membrane fatty acids were extracted with a mixture of chloroform and methanol (2:1, v:v), dissolved in heptane, and injected onto a capillary Varian CP7420 100-m column with a Hewlett Packard 5890 gas chromatograph. The gas chromatograph was configured for a single capillary column with a flame ionization detector and interfaced with HP chemstation software. Adequate separation of fatty acids methyl ester was obtained over a 50-min period with an initial temperature of 190 degrees C followed by increase to 240 degrees C. Fatty acids from 12:0 through 24:1(n-9) were separated and identified.(12) Laboratory CVs were 40.8% for t-16:1n-7, 3.8% for total t-18:1, 6.3% for c/t-18:2, 5.9% for t/c-18:2, and 32.5% for t/t-18:2. The concentration of each individual fatty acid (28 total identified) was expressed as a percentage of total area under the peaks.

Supplemental Material - References

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Supplemental Table 1. Mean values (percent of fatty acids) and Spearman correlation coefficients across individual trans fatty acids in the CARDIA cohort.

	CARDIA (n=1,507)						
	t-16:1n-7	**t-18:1n-6**	**t-18:1n-7 to n-9**	**total t-18:1**	**c/t-18:2 c/t**	**t/c-18:2**	**t/t-18:2**
Mean±SD	0.06±0.03	0.54±0.263	0.95±0.40	1.49±0.63	0.05±0.02	0.13±0.05	0.03±0.02
t-18:1n-6	0.37						
t-18:1n-7 to n-9	0.47	0.82					
total t-18:1	0.45	0.93	0.97				
c/t-18:2	0.22	0.31	0.39	0.38			
t/c-18:2	0.26	0.57	0.53	0.57	0.53		
t/t-18:2	0.22	0.33	0.50	0.45	0.28	0.14	

Supplemental Table 2. Mean values (percent of fatty acids) and Spearman correlation coefficients across individual trans fatty acids in the CHS cohort.

	CHS (n=2,404)									
	t-16:1n-7	**t-18:1n-6**	**t-18:1n-7**	**t-18:1n-8**	**t18:1n-9**	**t-18:1n-10 to n-12**	**total t-18:1**	**c/t-18:2**	**t/c-18:2**	**t/t-18:2**
Mean±SD	0.19±0.05	0.61±0.21	0.50±0.18	0.33±0.14	0.36±0.14	0.21±0.08	2.01±0.71	0.08±0.02	0.13±0.05	0.05±0.04
t-16:1n-7										
t-18:1n-6	0.12									
t-18:1n-7	0.24	0.93								
t-18:1n-8	0.20	0.88	0.86							
t-18:1n-9	0.00	0.94	0.86	0.85						
t-18:1n-10-12	0.06	0.91	0.82	0.85	0.91					
total t-18:1	0.14	0.98	0.95	0.93	0.95	0.93				
c/t-18:2	0.00	0.58	0.53	0.52	0.62	0.57	0.59			
t/c-18:2	0.00	0.49	0.47	0.46	0.50	0.49	0.51	0.71		
t/t-18:2	0.15	0.10	0.12	0.15	0.05	0.11	0.11	-0.09	0.00	

Supplemental Table 3. Mean values (percent of fatty acids) and Spearman correlation coefficients across individual trans fatty acids in the GOLDN cohort.

	GOLDN (n=1092)						
	t-16:1n-7	**t-18:1n-6**	**t-18:1n-7 to n-9**	**total t-18:1**	**c/t-18:2**	**t/c-18:2**	**t/t-18:2**
Mean±SD	0.07±0.03	0.34±0.11	1.06±0.32	1.40±0.42	0.09±0.02	0.10±0.03	0.042±0.02
t-16:1n-7							
t-18:1n-6	0.38						
t-18:1n-7 to n-9	0.41	0.90					
total t-18:1	0.41	0.95	0.99				
c/t-18:2	0.32	0.59	0.64	0.64			
t/c-18:2	0.34	0.50	0.53	0.53	0.80		
t/t-18:2	0.20	0.51	0.57	0.56	0.56	0.53	

Supplemental Table 4. Mean values (percent of fatty acids) and Spearman correlation coefficients across individual trans fatty acids in the HPFS cohort.

	HPFS (n=1295)				
	t-16:1n-7	**total t-18:1**	**c/t-18:2**	**t/c-18:2**	**t/t-18:2**
Mean±SD	0.14±0.04	1.50±0.64	0.12±0.05	0.08±0.04	0.01±0.02
t-16:1n-7					
total t-18:1	0.43				
c/t-18:2	0.18	0.57			
t/c-18:2	0.07	0.37	0.78		
t/t-18:2	0.04	0.14	0.15	0.12	

Supplemental Table 5. Mean values (percent of fatty acids) and Spearman correlation coefficients across individual trans fatty acids in the MESA cohort.

	MESA (n=707)						
	t-16:1n-7	**t-18:1n-6**	**t-18:1n-7 to n-9**	**total t-18:1**	**c/t-18:2**	**t/c-18:2**	**t/t-18:2**
Mean±SD	0.06±0.03	0.43±0.22	1.06±0.47	1.49±0.68	0.06±0.02	0.14±0.06	0.04±0.02
t-16:1n-7							
t-18:1n-6	0.58						
t-18:1n-7 to n-9	0.57	0.88					
total t-18:1	0.59	0.93	0.99				
c/t-18:2	0.26	0.55	0.54	0.55			
t/c-18:2	0.27	0.53	0.49	0.51	0.60		
t/t-18:2	0.19	0.50	0.55	0.54	0.36	0.41	

Supplemental Table 6. Mean values (percent of fatty acids) and Spearman correlation coefficients across individual trans fatty acids in the NHS cohort.

	NHS (n=655)				
	t-16:1n-7	**total t-18:1**	**c/t-18:2**	**t/c-18:2**	**t/t-18:2**
Mean±SD	0.15±0.04	1.62±0.71	0.18±0.06	0.13±0.05	0.09±0.06
t-16:1n-7					
total t-18:1	0.37				
c/t-18:2	0.25	0.80			
t/c-18:2	0.26	0.77	0.91		
t/t-18:2	-0.18	-0.38	-0.26	-0.31	

Supplemental Table 7. Mean values (percent of fatty acids) and Spearman correlation coefficients across individual trans fatty acids in the WGHS cohort.

	WGHS (n=652)				
	t-16:1n-7	**total t-18:1**	**c/t-18:2**	**t/c-18:2**	**t/t-18:2**
Mean±SD	0.06±0.03	1.79±0.53	0.09±0.02	0.10±0.05	0.03±0.01
t-16:1n-7					
total t-18:1	0.43				
c/t-18:2	0.28	0.69			
t/c-18:2	0.13	0.27	0.38		
t/t-18:2	0.19	0.61	0.41	0.18	

Supplemental Table 8. Single nucleotide polymorphisms (SNPs) that exceeded ($P \leq 5.0 \times 10^{-8}$) or approached ($P \leq 5.0 \times 10^{-6}$) genome-wide significance for associations with phospholipid trans fatty acid concentrations in meta-analysis of 7 European ancestry populations. [1]

Fatty acid	SNP	Allele1	Allele2	Allele1 frequency	Beta coefficient	Standard error	P value	Direction [2]
Trans-16:1n-7	rs2806949	a	g	0.3738	-0.0026	5.00E-04	1.04E-06	--+----
	rs2806946	a	t	0.366	-0.0026	5.00E-04	1.26E-06	-------
	rs1343607	a	g	0.3747	-0.0026	5.00E-04	1.68E-06	-------
	rs664461	t	c	0.5749	-0.0024	5.00E-04	2.00E-06	-------
	rs538679	a	c	0.4244	0.0024	5.00E-04	2.14E-06	+++++++
	rs2806971	a	g	0.37	-0.0025	5.00E-04	2.63E-06	--+----
	rs2246208	t	c	0.6303	0.0025	5.00E-04	2.78E-06	++-++++
	rs2806974	t	c	0.6291	0.0025	5.00E-04	2.95E-06	++-++++
	rs2806933	a	c	0.3791	-0.0025	5.00E-04	3.24E-06	-------
	rs7870863	a	c	0.0623	-0.0048	0.001	3.64E-06	-------
	rs13288988	t	c	0.0637	-0.0051	0.0011	3.66E-06	-?-----
	rs7986704	t	c	0.0562	-0.0051	0.0011	3.96E-06	-----+-
	rs8000124	a	g	0.564	0.0026	6.00E-04	3.99E-06	+++++-+
	rs7987251	t	c	0.0557	-0.0051	0.0011	4.10E-06	-----+-
	rs6986	c	g	0.2277	-0.0079	0.0017	4.74E-06	????-??
Total trans-18:1	rs12364177	a	t	0.9552	0.176	0.0363	1.21E-06	??+++++
	rs226444	a	g	0.4426	-0.0534	0.0114	2.73E-06	-------
	rs7325799	t	c	0.0199	0.1777	0.0386	4.27E-06	+++++++

	rs10097669	t	c	0.6734	-0.0461	0.0101	4.50E-06	-------
	rs4521954	a	g	0.295	-0.0476	0.0104	4.91E-06	-------
Cis/trans-18:2	rs174549	a	g	0.2918	0.0035	4.00E-04	4.77E-15	+++++++
	rs174548	c	g	0.7049	-0.0035	4.00E-04	4.90E-15	-------
	rs174555	t	c	0.7057	-0.0034	4.00E-04	6.34E-15	-------
	rs174556	t	c	0.294	0.0033	4.00E-04	2.98E-14	+++++++
	rs174576	a	c	0.3422	0.0032	4.00E-04	3.02E-14	+++++++
	rs174574	a	c	0.3355	0.0032	4.00E-04	3.18E-14	+++++++
	rs174577	a	c	0.3431	0.0032	4.00E-04	4.01E-14	+++++++
	rs1535	a	g	0.6659	-0.0032	4.00E-04	4.62E-14	-------
	rs174578	a	t	0.3447	0.0032	4.00E-04	4.80E-14	+++++++
	rs174550	t	c	0.6687	-0.0032	4.00E-04	5.61E-14	-------
	rs174547	t	c	0.6702	-0.0032	4.00E-04	6.17E-14	-------
	rs174546	t	c	0.3302	0.0032	4.00E-04	6.19E-14	+++++++
	rs174545	c	g	0.6702	-0.0031	4.00E-04	6.87E-14	-------
	rs174537	t	g	0.3296	0.0031	4.00E-04	7.96E-14	+++++++
	rs174536	a	c	0.6693	-0.0031	4.00E-04	1.20E-13	-------
	rs174535	t	c	0.6692	-0.0031	4.00E-04	1.31E-13	-------
	rs102275	t	c	0.6688	-0.0031	4.00E-04	1.91E-13	-------
	rs174583	t	c	0.3478	0.0031	4.00E-04	2.03E-13	+++++++
	rs174601	t	c	0.3731	0.0034	5.00E-04	2.51E-13	+++++++
	rs174541	t	c	0.6495	-0.0031	4.00E-04	4.45E-13	-------
	rs4246215	t	g	0.349	0.0031	4.00E-04	5.22E-13	+++++++

	rs174538	a	g	0.2955	0.0031	4.00E-04	1.28E-12	+++++++
	rs174528	t	c	0.632	-0.003	4.00E-04	4.42E-12	-------
	rs174534	a	g	0.6658	-0.0029	4.00E-04	3.90E-11	-------
	rs108499	t	c	0.3345	0.0029	4.00E-04	5.20E-11	+++++++
	rs509360	a	g	0.3148	-0.0031	5.00E-04	5.85E-10	----?--
	rs2072114	a	g	0.874	-0.0037	6.00E-04	3.13E-09	-------
	rs2524299	a	t	0.8756	-0.0037	6.00E-04	6.88E-09	-------
	rs2727270	t	c	0.1209	0.0038	6.00E-04	6.90E-09	+++++++
	rs2727271	a	t	0.8789	-0.0038	6.00E-04	7.14E-09	-------
	rs2845573	a	g	0.9173	-0.0044	8.00E-04	1.45E-08	-------
	rs2526678	a	g	0.0833	0.0043	8.00E-04	8.18E-08	+++++++
	rs2851682	a	g	0.9123	-0.0039	7.00E-04	1.26E-07	-------
	rs174570	t	c	0.1353	0.0032	6.00E-04	1.47E-07	+++++++
	rs10400317	t	c	0.5858	-0.0054	0.0011	7.64E-07	????-??
	rs174449	a	g	0.6452	-0.0021	4.00E-04	8.10E-07	-----+-
	rs3731714	t	c	0.2989	0.0022	5.00E-04	1.86E-06	+++++-+
	rs174448	a	g	0.6472	-0.002	4.00E-04	2.08E-06	-------
	rs16985452	t	g	0.9675	-0.0137	0.0029	2.63E-06	??--?--
	rs422249	t	c	0.3218	0.0021	4.00E-04	2.80E-06	+++++++
	rs174575	c	g	0.7453	-0.0021	5.00E-04	3.92E-06	-------
	rs1026153	a	g	0.5721	-0.002	4.00E-04	4.56E-06	-------
	rs1449672	t	c	0.4302	0.002	4.00E-04	4.75E-06	+++++-+
Trans/cis-18:2	rs16894446	t	c	0.026	0.0167	0.0033	2.74E-07	??+++++

	rs17099388	a	g	0.0344	-0.0127	0.0025	3.63E-07	-?-----
	rs11104877	t	g	0.8926	-0.0079	0.0016	3.83E-07	-?---+-
	rs11104850	a	g	0.1078	0.0078	0.0015	4.03E-07	+?+++-+
	rs6890562	a	t	0.9696	0.0145	0.0029	5.77E-07	+?++?++
	rs6830724	t	c	0.8499	-0.0067	0.0014	1.16E-06	?---?--
	rs11104688	t	c	0.8898	-0.0089	0.0018	1.36E-06	-?--?--
	rs6854292	a	g	0.8525	-0.0067	0.0014	1.74E-06	?---?--
	rs2116324	t	c	0.8277	-0.0066	0.0014	1.77E-06	?---?--
	rs11104803	c	g	0.9127	-0.0067	0.0014	3.01E-06	-------
	rs7914606	t	c	0.9392	-0.0077	0.0017	3.52E-06	-----+-
	rs6893858	c	g	0.9727	0.0143	0.0031	3.75E-06	+?++?++
Trans/trans-18:2	rs10469266	a	t	0.9837	0.007	0.0012	2.34E-08	+?-+?-+
	rs16958148	t	g	0.8375	0.002	4.00E-04	8.49E-08	+++++++
	rs12446301	t	c	0.1567	-0.0021	4.00E-04	2.18E-07	-------
	rs5752209	c	g	0.9844	-0.01	0.002	2.94E-07	??--?+-
	rs1399212	t	c	0.9553	0.0029	6.00E-04	3.07E-07	+++++--
	rs9934935	a	g	0.1645	-0.0018	3.00E-04	3.12E-07	-------
	rs11248534	t	c	0.229	-0.0023	5.00E-04	4.99E-07	-?-----
	rs10514550	t	c	0.8577	0.0018	4.00E-04	6.60E-07	+++++++
	rs7566684	a	g	0.981	-0.0082	0.0017	8.39E-07	???--+-
	rs6757720	c	g	0.981	-0.0082	0.0017	8.55E-07	???--+-
	rs488400	t	g	0.4161	0.0019	4.00E-04	1.01E-06	++++?-+
	rs4780141	t	c	0.0411	-0.0028	6.00E-04	1.05E-06	-----+-

rs6707470	a	g	0.9811	-0.008	0.0016	1.05E-06	???--+-
rs7181696	t	c	0.0407	-0.0028	6.00E-04	1.28E-06	-----+-
rs7174989	a	g	0.9595	0.0028	6.00E-04	1.29E-06	+++++-+
rs7952067	c	g	0.0738	0.0052	0.0011	1.49E-06	??++?++
rs2325695	a	g	0.9679	-0.0056	0.0012	2.11E-06	+?---+-
rs2325694	a	g	0.0322	0.0056	0.0012	2.24E-06	+?+++-+
rs6670062	t	c	0.0218	-0.0046	0.001	2.28E-06	-?--+--
rs8184969	t	c	0.0153	0.0087	0.0019	2.34E-06	??++--+
rs4780144	t	c	0.961	0.0027	6.00E-04	2.39E-06	+++++-+
rs361171	t	c	0.5657	0.0014	3.00E-04	2.47E-06	++-+--+
rs5752223	t	c	0.0153	0.0087	0.0019	2.56E-06	??++--+
rs13103223	t	c	0.9142	0.0023	5.00E-04	2.58E-06	+++++++
rs10014313	t	c	0.9143	0.0023	5.00E-04	2.75E-06	+++++++
rs6821057	c	g	0.9144	0.0023	5.00E-04	2.75E-06	+++++++
rs6821987	c	g	0.0855	-0.0023	5.00E-04	2.85E-06	-------
rs4517515	t	c	0.1358	-0.0028	6.00E-04	2.99E-06	-?+-?--
rs2376264	c	g	0.9143	0.0022	5.00E-04	3.08E-06	+++++++
rs945631	a	g	0.0302	-0.0057	0.0012	3.10E-06	-?--?+-
rs16958145	t	c	0.1086	-0.0021	4.00E-04	3.19E-06	-------
rs4517514	t	c	0.1359	-0.0028	6.00E-04	3.35E-06	-?+-?--
rs6836899	t	c	0.0857	-0.0022	5.00E-04	3.35E-06	-------
rs6836750	a	c	0.0858	-0.0022	5.00E-04	3.36E-06	-------
rs6836250	a	g	0.0863	-0.0021	5.00E-04	3.42E-06	-------
rs10030764	t	c	0.0867	-0.0021	5.00E-04	3.43E-06	-------

rs2174496	a	g	0.9135	0.0021	5.00E-04	3.47E-06	+++++++
rs10031224	t	c	0.0866	-0.0021	5.00E-04	3.59E-06	-------
rs2376265	a	g	0.0863	-0.0023	5.00E-04	3.83E-06	-------
rs7649275	a	g	0.8491	0.0016	3.00E-04	3.85E-06	+++++++
rs1394866	a	g	0.0941	-0.002	4.00E-04	3.95E-06	-------
rs13149971	t	c	0.9154	0.0021	5.00E-04	3.96E-06	+++++++
rs2633727	a	g	0.1534	-0.0016	4.00E-04	4.01E-06	-------
rs10034736	t	c	0.0934	-0.002	4.00E-04	4.16E-06	-------
rs2612034	t	c	0.151	-0.0016	3.00E-04	4.22E-06	-------
rs1020820	a	g	0.8504	0.0016	3.00E-04	4.24E-06	+++++++
rs9714717	a	g	0.0866	-0.0021	4.00E-04	4.32E-06	-------
rs2680664	a	g	0.1501	-0.0016	3.00E-04	4.42E-06	-------
rs7672520	t	g	0.9124	0.002	4.00E-04	4.48E-06	+++++++
rs2169143	c	g	0.1522	-0.0016	3.00E-04	4.58E-06	-------
rs1020821	a	g	0.1532	-0.0016	3.00E-04	4.71E-06	-------
rs7694110	a	g	0.911	0.002	4.00E-04	4.79E-06	+++++++
rs9992966	t	c	0.0899	-0.002	4.00E-04	4.79E-06	-------
rs10012750	a	g	0.9098	0.002	4.00E-04	4.80E-06	+++++++
rs10034852	t	c	0.0909	-0.002	4.00E-04	4.82E-06	-------
rs2469054	a	g	0.0211	-0.0053	0.0012	4.82E-06	----?+-

[1] CARDIA (n=1,507), CHS (n=2,404), GOLDN (793), HPFS (n=1,295), MESA (n=707), NHS (n=655), and WGHS (652).

[2] Each symbol denotes the direction of association in each of the 7 cohorts; "?" denotes no SNP data available.

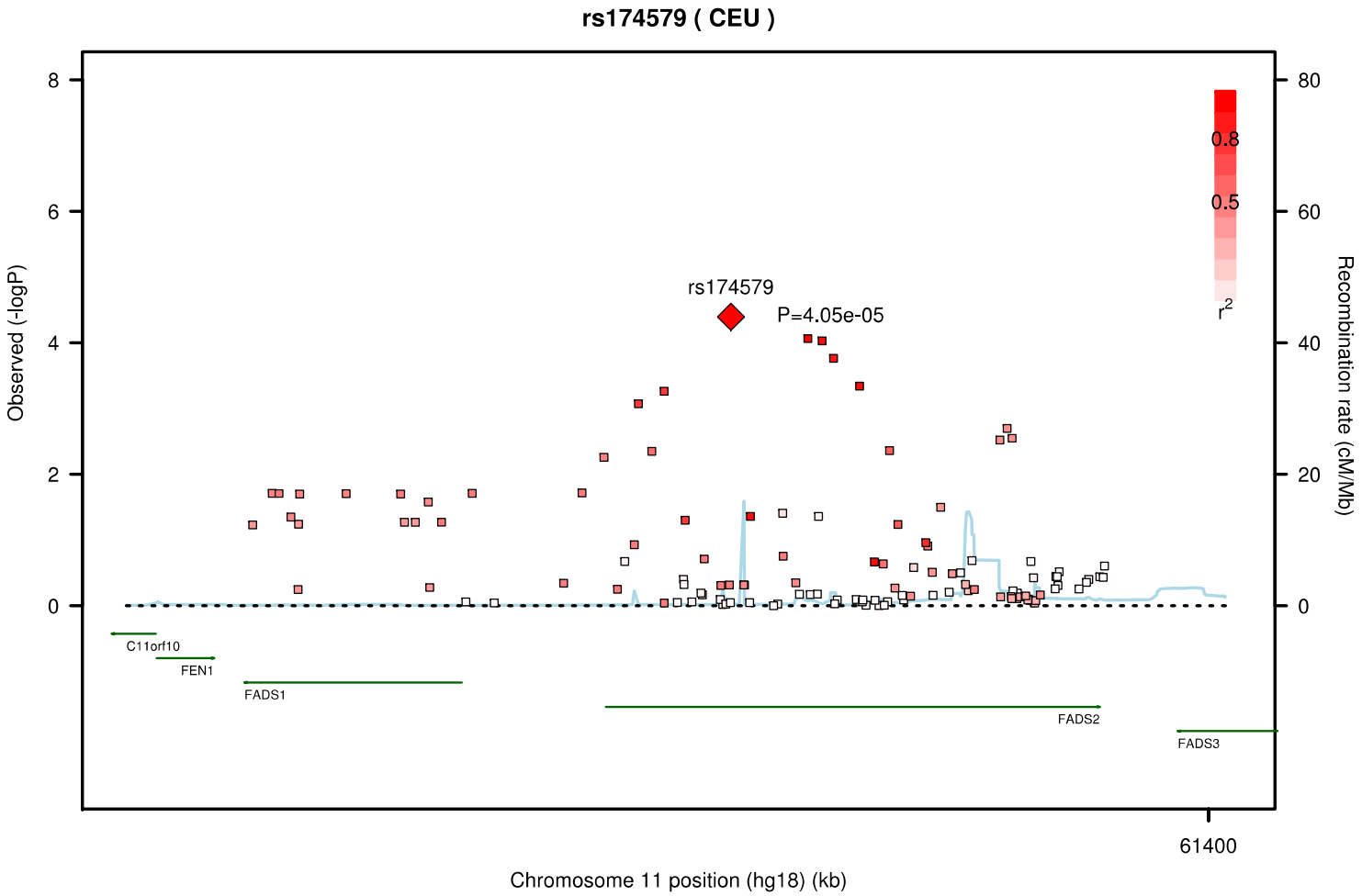
Supplemental Table 9. Associations between the 8 top SNPs that approached genome-wide significance ($P<5.0x10^{-7}$) and phospholipid trans fatty acid concentrations in non-European ancestry populations. [1]

Chromosome	SNP	Coded allele	Fatty acid	Population	Coded allele frequency	Beta coefficient (SE)	P value
5	rs16894446	T	trans/cis-18:2	CHS AA	N/A	--	--
				MESA AA	0.01	0.0149 (0.0134)	0.26
				MESA HA	0.13	0.0015 (0.0016)	0.36
				MESA CHA	0.35	0.0026 (0.0018)	0.14
5	rs17099388	A	trans/cis-18:2	CHS AA	0.24	0.0041 (0.0039)	0.29
				MESA AA	0.25	-0.0003 (0.0032)	0.94
				MESA HA	0.18	0.0010 (0.0014)	0.45
				MESA CHA	0.51	-0.0003 (0.0017)	0.89
12	rs11104877	G	trans/cis-18:2	CHS AA	N/A	--	--
				MESA AA	0.02	-0.0030 (0.0117)	0.80
				MESA HA	0.06	-0.0053 (0.0026)	0.04
				MESA CHA	N/A	--	--
2	rs7566684	G	trans/trans-18:2	CHS AA	0.25	0.0053 (0.0040)	0.19
				MESA AA	0.23	-0.0004 (0.0015)	0.80
				MESA HA	0.04	0.0005 (0.0028)	0.86
				MESA CHA	N/A	--	--
4	rs1399212	C	trans/trans-18:2	CHS AA	N/A	--	--
				MESA AA	N/A	--	--
				MESA HA	0.02	0.0002 (0.0045)	0.96
				MESA CHA	N/A	--	--

Chromosome	SNP	Coded allele	Fatty acid	Population	Coded allele frequency	Beta coefficient (SE)	P value
10	rs11248534	T	trans/trans-18:2	CHS AA	0.43	-0.0042 (0.0025)	0.10
				MESA AA	0.44	0.0015 (0.0013)	0.25
				MESA HA	0.27	-0.0002 (0.0015)	0.88
				MESA CHA	0.46	0.0022 (0.0012)	0.08
16	rs16958148	G	trans/trans-18:2	CHS AA	0.08	0.0129 (0.0143)	0.37
				MESA AA	0.10	-0.0024 (0.0021)	0.27
				MESA HA	0.20	0.0014 (0.0016)	0.37
				MESA CHA	0.26	0.0004 (0.0014)	0.79
22	rs5752209	G	trans/trans-18:2	CHS AA	N/A	--	--
				MESA AA	N/A	--	--
				MESA HA	0.07	-0.0034 (0.0028)	0.22
				MESA CHA	0.11	-0.0039 (0.0021)	0.07

[1] Replication of rs10469266, which achieved nominal genome-wide significance among European participants, was evaluated at two-tailed alpha=0.05. Each of the other 8 SNPs was considered a secondary exploratory hypothesis, evaluated at two-tailed alpha=0.05/8 = 0.00625.

AA=African Americans (N=446 in CHS, N=637 in MESA); HA=Hispanic Americans (N=657); CHA=Chinese Americans (N=669); CHS=Cardiovascular Health Study; MESA=Multi-Ethnic Study of Atherosclerosis. N/A=SNP or fatty acid data not available.



Supplemental Figure 1. Regional association plot for cis/trans-18:2, based on meta-analysis of fine mapping of *FADS1* and *FADS2* (build 37: 61566604-61635303; ~70kb) in African Americans in CHS (N=445) and MESA (N=637).