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ARTICLE

Genome-wide association study reveals shared and distinct genetic architecture of fatty acids and oxylipins in the Hispanic Community Health Study/Study of Latinos

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Summary

Bioactive fatty acid-derived oxylipin molecules play key roles mediating inflammation and oxidative stress. Circulating levels of fatty acids and oxylipins are influenced by environmental and genetic factors; characterizing the genetic architecture of bioactive lipids could yield new insights into underlying biology. We performed a genome-wide association study (GWAS) of 81 fatty acids and oxylipins in 11,584 Hispanic Community Health Study/Study of Latinos (HCHS/SOL) participants with genetic and lipidomic data measured at study baseline (58.6% female, mean age = 46.1 years (standard deviation 13.8)). Additionally, given the effects of central obesity on inflammation, we examined interactions with waist circumference using two-degree-of-freedom joint tests. Thirty-three of the 81 oxylipins and fatty acids were significantly heritable (heritability range: 0–32.7%). Forty (49.4%) oxylipins and fatty acids had at least one genome-wide significant ($p < 6.94E{-}11$) variant resulting in 19 independent genetic loci. Six loci (lead variant minor allele frequency [MAF] range: 0.08–0.50), including desaturase-encoding *FADS* and OATP1B1 transporter protein-encoding *SLCO1B1*, exhibited associations with two or more fatty acids and oxylipins. At several of these loci, there was evidence of colocalization of the top variant across fatty acids and oxylipins. The remaining loci were only associated with one oxylipin or fatty acid and included several *CYP* loci. We also identified an additional rare variant (MAF = 0.002) near *CARS2* in two-degree-of-freedom tests. Our analyses revealed shared and distinct genetic architecture underlying fatty acids and oxylipins, providing insights into genetic factors and motivating work to characterize these compounds and elucidate their roles in disease.

Introduction

Chronic inflammation is a pathological feature underlying many chronic diseases,[1–5] and inflammatory responses involve a variety of so-called "inflammatory mediators" that include both signaling proteins (e.g., cytokines, chemokines) and bioactive lipids such as oxylipins.[6] Oxylipins are derived from the oxygenation of precursor mono- and polyunsaturated fatty acids through cascading pathways mediated by cytochrome P450 (CYP), lipoxygenase (LOX), and cyclooxygenase (COX) enzymes, or through other enzymatic and non-enzymatic reactions[7] (Figure S1), and play a variety of roles mediating inflammation and oxidative stress.[8,9] Given the role of inflammation in many chronic diseases, there is growing interest in oxylipin-targeted therapies.[10] Oxylipins and their precursor fatty acids are influenced by both environmental and genetic factors.[11–13] Thus, characterizing the genetic architecture of oxylipins and related fatty acids could yield new insights into underlying biological pathways that could inform drug targets.[11]

Genetic analyses of several polyunsaturated fatty acids from which many oxylipins are derived have identified multiple variants at loci encoding desaturase and elongase enzymes (e.g., *FADS, ELOVL2*).[14–18] Moreover, previous associations between the LOX-encoding *ALOX12* locus and 12-HETE oxylipins have been reported, consistent with the role that LOX enzymes play in the derivation of these metabolites.[12,19] Still, few analyses of the genetic architecture of oxylipins have been conducted on a large scale. This is due in part to historical challenges in quantifying and annotating oxylipin species: hundreds of oxylipins with distinct structures and stereochemistry can be derived from fatty acids.[8] However, recent advancements in mass spectrometry-based metabolomics and annotation approaches have enabled the

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improved detection, quantification, and annotation of oxylipins.[20–22]

To address this research gap, we estimated the heritability and performed a genome-wide association study (GWAS) of $n = 81$ oxylipins and related fatty acids quantified via directed, non-targeted liquid chromatography-mass spectrometry (LC-MS) in the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). Moreover, motivated by the fact that central adiposity is associated with other markers of low-grade inflammation (e.g., C-reactive protein and interleukin-6)[23–26] and may influence oxylipin biosynthesis,[27] we also extended our models to include interactions with waist circumference and performed two-degree-of-freedom joint tests, increasing our power to identify additional loci that have both main and interaction effects.[28,29] Our findings reveal both shared and distinct genetic architecture of modest-to-moderately heritable circulating fatty acids and oxylipins, providing insights into the biological pathways underlying these metabolites.

Materials and methods

Hispanic community health study/study of latinos (HCHS/SOL)

HCHS/SOL is a multi-center, community-based cohort study of Hispanic/Latino adults in the United States designed to study the incidence and prevalence of, and risk factors for, multiple chronic diseases, including cardiovascular diseases, kidney, lung and liver diseases.[30] A total of 16,415 participants of Mexican, Puerto Rican, Dominican, Cuban, and Central and South American background aged 18–74 years were recruited from four U.S. cities between 2008 and 2011: Bronx, NY; Chicago, IL; Miami, FL; and San Diego, CA. Households were selected via two-stage sampling within census block groups.[31] At study baseline (2008–2011), participants received interviewer-administered questionnaires and standardized examinations, including biospecimen collection. Informed consent was obtained from all participants, and the study was conducted in accordance with the principles of the Declaration of Helsinki. Approval from the University of North Carolina at Chapel Hill Institutional Review Board was obtained for this work.

Oxylipin profiling, annotation, and quality control

Oxylipin profiling of fasting plasma samples collected at HCHS/SOL study baseline was conducted by Sapient Bioanalytics, using a rapid liquid chromatography-mass spectrometry (rLC-MS) methodology that has been described in detail previously[22] (supplemental methods). Oxylipins and fatty acids were annotated by aligning experimental accurate mass-to-charge ratio (m/z), retention time (RT) and MS2 spectra with an in-house curated library of commercial standards as well as alignment with previously reported structural isomers.[1] These annotations often indicated multiple potential chemically related compound mappings, due to compounds with similar chemical formulas and mass-to-charge ratios co-eluting at the same time. Furthermore, through chemical networking of spectral data from nontargeted data collection, additional members of the oxylipin family were identified.[21] Oxylipin metabolites with chemical structures that aligned with previously reported structural isomers for which biological pathways were unknown were annotated as "putative known oxylipins." Likewise, oxylipins lacking database entries in LipidMaps or the Human Metabolome Database (https://hmdb.ca), but exhibiting spectral fragmentation patterns highly similar to those of known oxylipins were annotated as "putative novel oxylipins." Their exact identities cannot be determined without further investigation, beyond the scope of this work.

In this study, we included $n = 81$ metabolites annotated as oxylipins and fatty acids based on the procedure described above. To facilitate interpretation of analyses, for the oxylipin metabolites, we defined precursor fatty acid groupings based on literature searches describing the biosynthetic pathways of oxylipin compounds as well as online resources such as PubChem (https://pubchem.ncbi.nlm.nih.gov), the Human Metabolome Database, and Cayman Chemical (https://www.caymanchem.com). When annotations indicated multiple potential compound mappings due to compounds with similar chemical formulas and mass-to-charge ratios co-eluting at the same time, we grouped them based on any of the precursor fatty acids from which they may have been derived (e.g., "arachidonic acid or eicosapentaenoic acid"). Moreover, fatty acids were grouped based on the class of fatty acids to which they belonged: polyunsaturated fatty acids, monounsaturated fatty acids, or saturated fatty acids.

The percent missingness across all 81 metabolites ranged from 0% to 23.4%, with the majority of metabolites (93.8%) missing less than 2% of all observations. Missing values in the metabolite data reflect values below the lower limit of detection of the measurement instruments. Therefore, to mimic low end values for metabolites with values below the limit of detection, missing metabolite observations were imputed from a random uniform distribution between the minimum measured metabolite value/10 and the minimum measured metabolite values. All metabolite values were subsequently $\log_2$-transformed to reduce the impact of extreme outlier values.[32]

Genotyping and quality control

HCHS/SOL participants were genotyped on the Infinium Expanded Multi-Ethnic Genotyping Array (MEGA),[33] and imputed to the TOPMed R2 (Freeze 8) imputation panel using IMPUTE v2.3.2 (supplemental methods). Variants with an imputation quality score <0.4 or effective sample size <20, calculated as $2\times MAF\times(1\text{-}MAF)\times N\times Q$, where MAF is minor allele frequency, Q is imputation quality, and N is sample size, were excluded from main effect analyses. For joint effect analyses, an effective sample size filter of 30 was applied, where the N used in effective sample size calculations was the sex-stratified sample size ($N_{females} = 6725$, $N_{males} = 4753$). All genomic coordinates are reported on GRCh38.

Spearman correlations

Because Spearman correlations are more robust to outliers and non-normal distributions than Pearson correlations,[34] we computed Spearman correlations of the $n = 81$ oxylipins and fatty acids to determine their correlation structure.

Heritability analysis

We estimated SNP-based heritability, or the proportion of variance explained for each metabolite due to the additive effect of genotyped common variants, and corresponding 95% confidence intervals, using Haseman-Elston regression, as previously described for the HCHS/SOL study.[35] For this analysis, we used a kinship matrix calculated using autosomal variants that were genotyped

on the Infinium Expanded Multi-Ethnic Genotyping Array (MEGA),[33] and imputed to the TOPMed R2 (Freeze 8) imputation panel using IMPUTE v2.3.2.

Main effects GWAS

The $\log_2$-transformed metabolite values were regressed on age, age,[2] sex, study recruitment site, and self-identified background, and residuals from these models were subsequently inverse rank normalized to yield asymptotically normal marginal distributions.[36] Genetic association analyses of the inverse rank normalized residuals were conducted using generalized estimating equations via SUGEN,[37] adjusting for 10 ancestral principal components, which were calculated using Plink. Results after genomic control correction are reported.

To account for multiple testing of 81 correlated oxylipins and fatty acids, we used a modified Bonferroni correction based on the effective number of independent tests.[38] Briefly, we computed the number of principal components necessary to explain 99.5% of metabolite variability ($N_{effective}$ = 72), yielding a multiple testing-corrected p-value threshold of 5E−09/72 = 6.94E−11. We used EasyStrata software[39] to identify independent loci and corresponding top variants, using 500 kB as the physical position threshold for clumping. We used dbSNP (https://www.ncbi.nlm.nih.gov/snp/) and the Ensembl Variant Effect Predictor tool (https://useast.ensembl.org/info/docs/tools/vep/index.html)[40] to annotate the nearest genetic locus and identify the predicted consequence of each of our top variants.

Main effects conditional analyses

We performed stepwise exact conditional analyses to identify additional independent secondary signals for each genome-wide significant locus identified in the main effects GWAS. Briefly, we included the top genome-wide significant variant at each significant locus as a covariate in our SUGEN analysis and iterated until no novel significant secondary signals remained. Because conditional analyses are not performed on a genome-wide scale but rather are limited to genomic regions surrounding primary GWAS index signals, applying a genome-wide significance level for conditional analyses is too strict and can lead to loss of power.[41] Therefore, as a conservative approach, we defined significant secondary signals as variants reaching a p-value threshold one order of magnitude above our genome-wide threshold ($p < 6.94E{-}10$). Finally, we estimated the proportion of variance in the phenotype explained (PVE) by a given variant for each independent index and secondary variants using the following equation, as previously described[42,43]:

$$PVE = \frac{2\widehat{\beta}^2 MAF(1 - MAF)}{2\widehat{\beta}^2 MAF(1 - MAF) + se(\widehat{\beta})^2 2NMAF(1 - MAF)}$$

Bayesian co-localization analysis

For genetic loci associated with two or more oxylipins or fatty acids, we used the 'coloc' R package (version 5.2.3) to estimate the probability of each pair of associated metabolites sharing a common causal variant.[44] More details are provided in the supplemental methods.

Joint effects GWAS

To increase the power to identify additional loci in which genetic variants exhibit both a main effect and an interaction effect,[28,29] we also conducted joint two-degree-of-freedom tests accounting for waist circumference as a measure of central adiposity. Because sex-specific waist circumference thresholds have been recommended for defining central obesity,[45,46] we conducted sex-stratified joint effect analyses, including inverse rank normalized residuals of waist circumference adjusted for body mass index (BMI) as an interaction term in our analyses. Waist circumference, weight, and height were measured using standardized protocols at the baseline study visit. Waist circumference adjusted for BMI was calculated in sex-stratified analyses, regressing waist circumference on age, age,[2] and BMI, and then inverse rank normalizing the residuals.[47] We then meta-analyzed the p-values for the joint variant and variant x waist circumference effect ("joint p-value") using METAL,[48] weighting by sample size.

All figures were generated using R version 4.1.0, using the colorblind-friendly color palette from the following resource: https://zenodo.org/record/3381072.

Results

There were n = 11,610 SOL participants with measured genetic and metabolite data at the baseline visit. After excluding n = 26 participants with missing self-reported background group, the final main effects analysis sample was n = 11,584. This analytic sample was 58.6% female, and the mean age of participants was 46.1 years (standard deviation [SD] = 13.8). After excluding an additional n = 106 with missing BMI-adjusted-waist circumference, the final joint effect analysis sample was $n_{Females}$ = 6,725 and n_{Males} = 4,753 (total n = 11,478). The mean waist circumference for females was 97.6 cm (SD = 14.2 cm), and the mean waist circumference for males was 99.0 cm (SD = 13.3 cm).

Spearman correlation of fatty acids and oxylipins

Spearman correlations of the $\log_2$-transformed values of the 81 oxylipins and fatty acids ranged between −0.27 and 0.98 (mean = 0.21), with the strongest correlations observed between fatty acids (range: −0.22 to 0.90, mean = 0.34) (Figures 1 and S2). Among the oxylipins, correlations varied both within and between precursor fatty acid groupings and were highest for oxylipins derived from linoleic acid (range: −0.12 to 0.70, mean = 0.28) and lowest for oxylipins putatively derived from arachidonic acid or eicosapentaenoic acid (range: −0.06 to 0.18, mean = 0.07) (Figures 1 and S2).

Heritability of fatty acids and oxylipins

Thirty-three of the 81 (40.7%) fatty acids and oxylipins exhibited significant ($p < 0.05$) heritability (significant h_{SNP}^2 range: 4.95%–32.74%), the magnitude of which varied across the 34 fatty acids and 47 oxylipins (Table S1). Among metabolites with significant heritability, fatty acids generally exhibited higher heritability (mean h_{SNP}^2 = 12.01%, SD = 7.56%, range: 5.74%–32.74%) than oxylipins (mean h_{SNP}^2 = 7.61%, SD = 2.61%, range: 4.95%–16.07%) (Figure 2; Table S1). Among

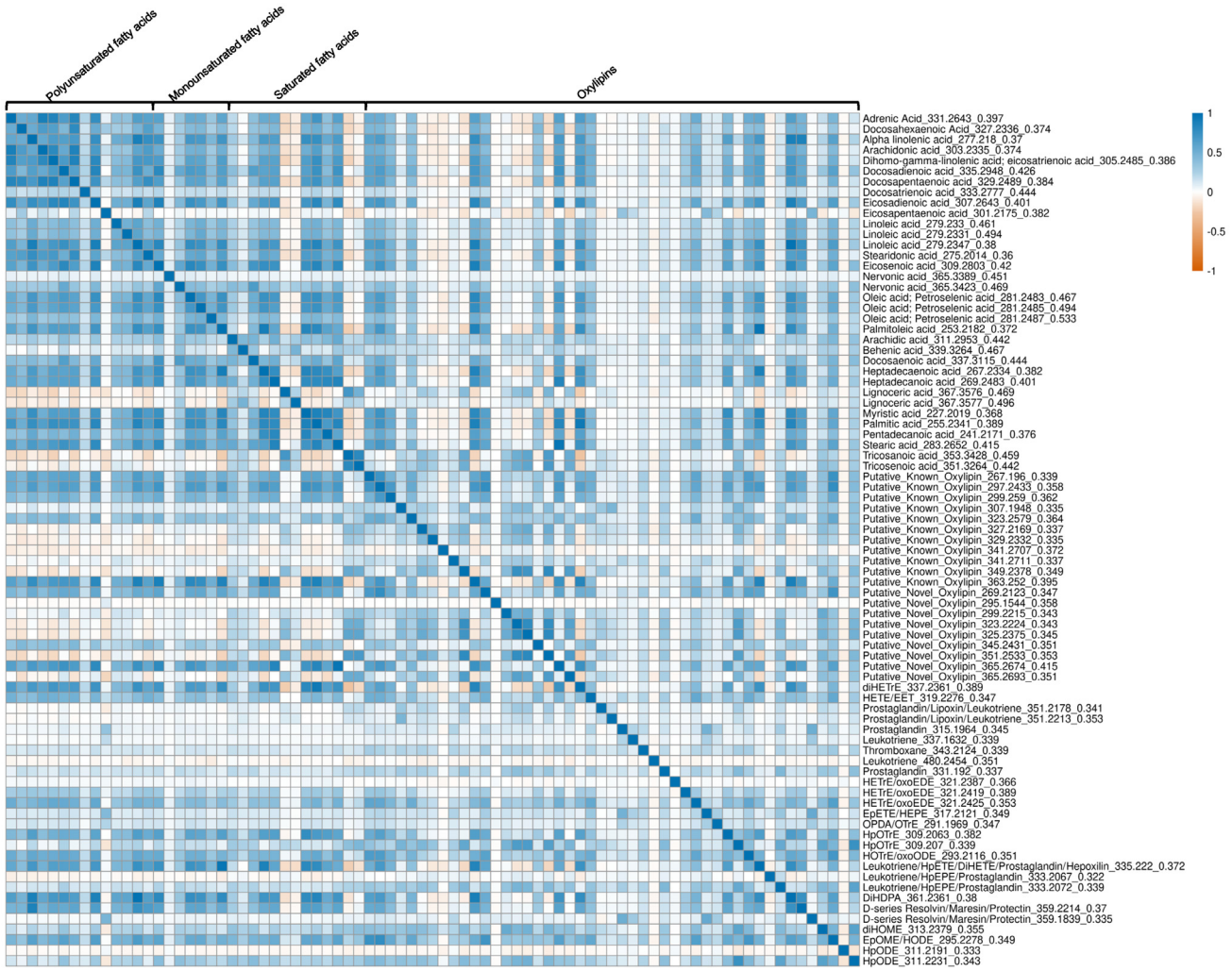

Figure 1. Spearman correlation matrix of log2-transformed values of $n = 81$ putative fatty acids and oxylipin metabolites

Positive correlations are displayed in blue, negative correlations are displayed in orange. Metabolites are annotated with the putative general class of compound to which they belong, their measured mass-to-charge ratio (m/z), and their retention time (RT) in the form: metabolite name_m/z_RT. When annotations indicated multiple potential compound mappings due to compounds with similar chemical formulas and mass-to-charge ratios co-eluting at the same time, we have listed all of them, separated by backslashes (e.g., HETE/EET). Metabolites are ordered in the matrix based on these general groupings, with putative free fatty acids first (polyunsaturated fatty acids, monounsaturated fatty acids, saturated fatty acids), followed by oxylipin metabolites.

oxylipins, heritability estimates were generally consistent across fatty acid precursor groups.

Main effects GWAS

Of the 81 fatty acids and oxylipins, 40 (49.4%) had at least one genome-wide significant ($p < 6.94E{-}11$) variant. These metabolites included 26 of the 33 (78.8%) significantly heritable fatty acids and oxylipins (Table S1). Additionally, 15 of the 48 (31.3%) fatty acids and oxylipins that were not significantly heritable also exhibited genome-wide significant associations. After accounting for variants that mapped to the same locus, there were 19 independent loci, and 55 unique metabolite-locus pairs (Figure 3A; Table S2; Figure S3). There were also 12 highly correlated metabolite pairs (Spearman correlation coefficient >0.80) for which one metabolite had a genome-wide significant association, but the other did not have a genome-wide significant, or near genome-wide significant association, for the same locus (see Table S3). The top variants were all common (minor allele frequency (MAF) > 0.01). As previously reported, allele frequencies for several of the loci we identified varied across reference continental ancestry populations, including *FADS*,[18] at which the MAF of nearly all of the top variants was highest in our HCHS/SOL sample compared to reference populations, the *CYP* supergene family,[49] *SLCO1B1*,[50,51] *FUT2*,[52] and *MCM6*.[53] Moreover, *ADH1A* top variant rs28600890 was monomorphic in 1000 Genomes European and South and East Asian reference populations (Table S2; Figure S4).

Shared genetic architecture

The genetic architecture of the $n = 40$ fatty acids and oxylipins with at least one genome-wide significant variant was characterized by both shared and distinct

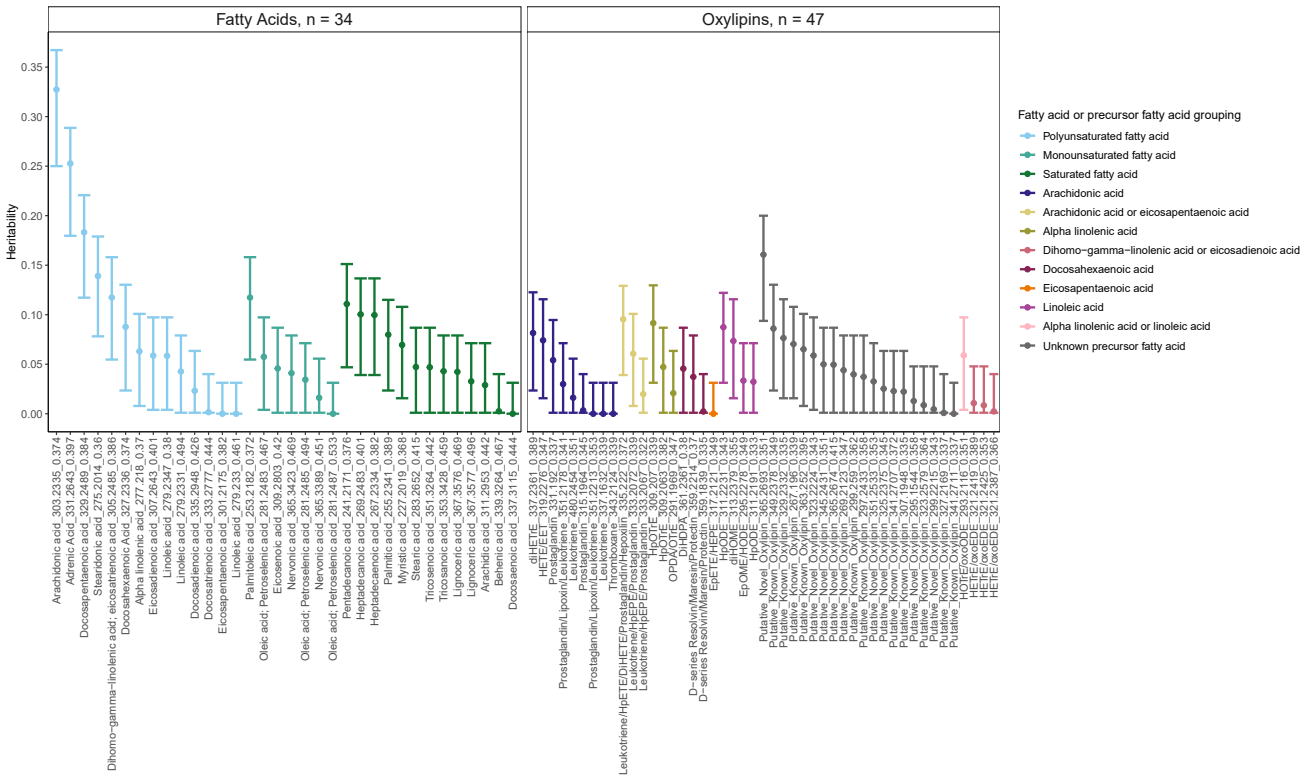

Figure 2. Estimated proportion of variance due to kinship (heritability) and 95% confidence intervals of $n = 81$ putative fatty acids and oxylipin metabolites

Metabolites are annotated with the putative general class of compound to which they belong, their measured mass-to-charge ratio (m/z), and their retention time (RT) in the form: metabolite name_m/z_RT. Estimates are colored by the putative grouping to which the metabolite belongs: polyunsaturated fatty acid, monounsaturated fatty acid, saturated fatty acid, or the putative fatty precursor from which the putative oxylipin is derived. Putative novel and known oxylipins are grouped in "Unknown precursor fatty acid" grouping.

genetic effects. Of the 19 identified loci, six pleiotropic loci (*SLCO1B1, FADS, PKD2L1, PPP1R3B, CYP4F2,* and the locus block including *ACOT2* and *ACOT6*) were associated with at least two metabolites (Figure 3B), accounting for 42 (76.4%) of the 55 unique metabolite-locus associations. Only oxylipins, including several putatively novel oxylipins, were associated with the *SLCO1B1*, *CYP4F2*, and *ACOT* loci. In contrast, consistent with its known biological roles,[14] the majority of associations with the *FADS* locus were for polyunsaturated fatty acids, while *PKD2L1* and *PPP1R3B* were associated with several saturated or monounsaturated fatty acids, as well as oxylipins (Figure 3B). Spearman correlations among the sets of metabolites associated with these shared pleiotropic loci varied (range in mean correlations: 0.18–0.74) (Table S4).

Top variants at these loci explained the highest estimated percentages of trait variance of all the metabolite-locus associations. For example, *FADS* variant rs174564 explained 9.4% of arachidonic acid (m/z = 303.23, RT = 0.374) trait variance and *SLCO1B1* variant rs4149056 explained 6.78% of HpODE (m/z = 311.22, RT = 0.343) trait variance (Table S2). In conditional analyses, significant secondary signals were identified at three of these pleiotropic loci, all of which explained a lower percentage of variance compared to the top index variant: for eight of the *SLCO1B1*-associated oxylipins (top secondary variants: rs11045856, rs77271279, and rs59502379, percent variance range: 0.36–2.21%), for the arachidonic acid-*FADS* association (top secondary variant rs174570, percent variance: 0.65%), and for the putative novel oxylipin associated with *CYP2C9* (top secondary variant rs1919 91016, percent variance: 0.42%) (Table S5).

Colocalization

These pleiotropic loci showed little evidence of allelic heterogeneity (Tables S2 and S5), with the same top variant (and secondary signals, when identified) shared by many or all of the associated metabolites. These top variants included two missense variants (*SLCO1B1*-rs4149056 and *CYP4F2*-rs2108622). Colocalization analyses revealed strong evidence of colocalization ($P_{H4} \geq 0.80$) for all pairwise combinations of associated metabolites for the *PPP1R3B*, *PKD2L1*, *SLCO1B1*, and *CYP4F2* loci (Tables S5–S9). There was no evidence of colocalization for the two oxylipins associated with variants near the *ACOT* gene cluster ($P_{H4} = 2.26E{-}11$, $P_{H3} = 0.999$), consistent with the fact that the top variants' nearest genes are different genes (*ACOT2* and *ACOT6*) within the *ACOT* gene cluster (Table S10). At the *FADS* locus, colocalization

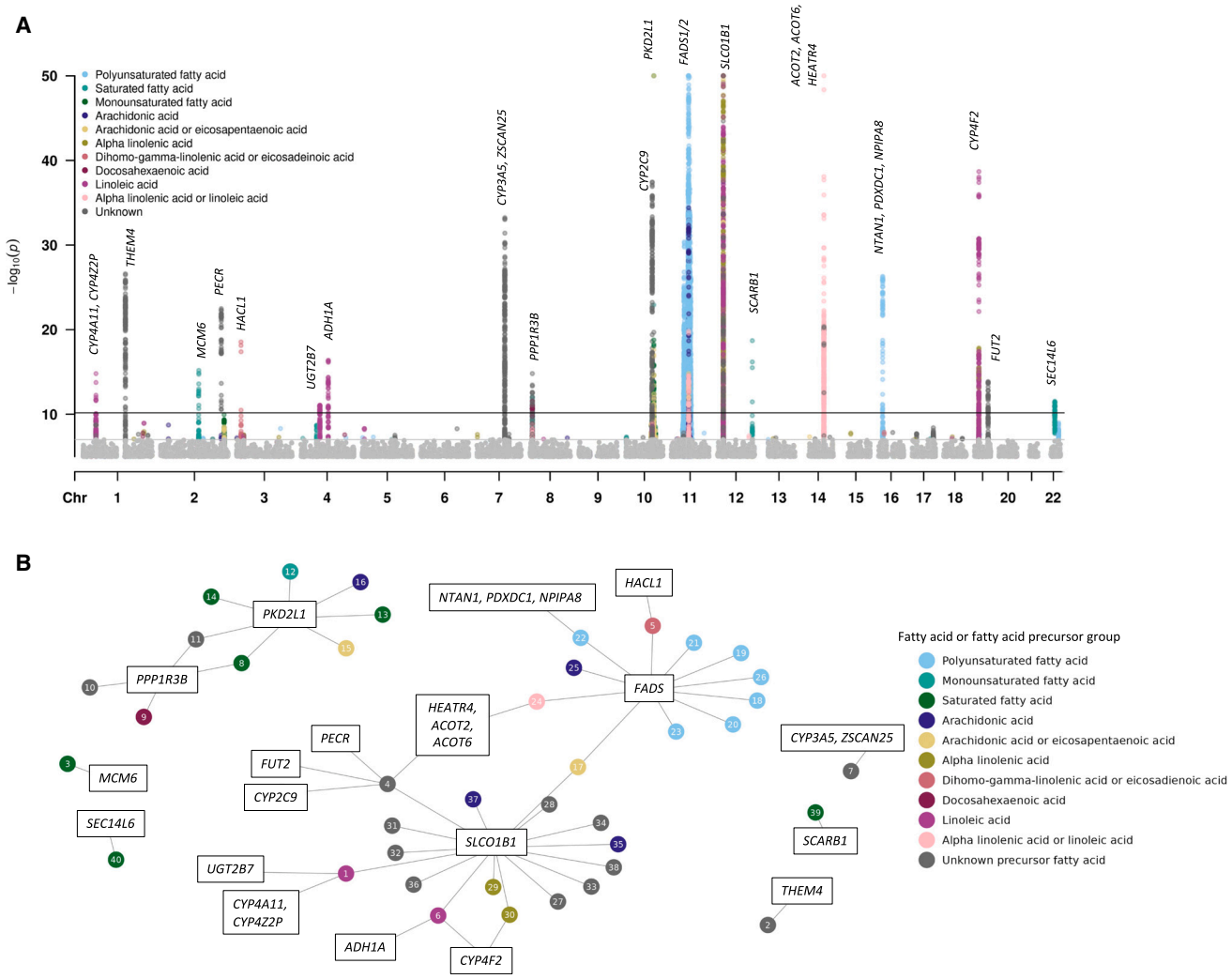

Figure 3. Summary of unique loci identified in main effects GWAS

(A) Manhattan plot of unique loci identified in main effects GWAS, color coded by the putative grouping to which the metabolite belongs: polyunsaturated fatty acid, monounsaturated fatty acid, saturated fatty acid, or the putative fatty precursor from which the putative oxylipin is derived. Oxylipins annotated as 'putative novel' or 'putative known' are colored in gray, and precursor fatty acid group is marked as "Unknown precursor fatty acid." -log10 *p*-values were capped at 50 to aid visualization of results (maximum -log10pvalue observed was 262, for arachidonic acid associated with *FADS2*). Some annotations included multiple putative compounds which are derived from different fatty acid precursors, so both are listed (e.g., dihomo-gamma-linolenic acid or eicosadienoic acid; arachidonic acid or eicosapentaenoic acid). Black line indicates genome-wide significance threshold (5E−09/72 = 6.94E−11); all variants with *p*-value >1E−7 are colored in gray to improve visualization of results.

(B) Network plot of the 56 unique metabolite-locus associations to illustrate pleiotropic and distinct genetic effects. Genetic loci are in boxed labels, metabolites are colored based on the putative fatty acid or precursor fatty acid grouping from which the oxylipin is derived. Metabolites are numbered (1–40) and these numbers correspond to the "metabolite id number" column in Table S2.

patterns varied by pairs of metabolites (Table S11; Figure S5) For example, dihomo-gamma-linolenic acid (m/z = 305.2485, RT = 0.386) did not exhibit strong evidence of colocalization with any of the other *FADS*-associated metabolites, while docosahexaenoic acid (m/z = 327.2336, RT = 0.374) exhibited strong evidence of colocalization with eight of the other *FADS*-associated metabolites.

Distinct genetic architecture

The remaining 13 significant loci were each associated with only a single metabolite (*MCM6; SCARB1; SEC14L6; THEM4; CYP3A5, ZSCAN25; ADH1A; FUT2; PECR; CYP2C9; HACL1; NTAN1, PDXDC2, NPIPA8; UGT2B7; CYP4A11, CYP4Z2P*) (Figure 3B; Table S2). Nine of these 13 loci were associated with oxylipins (*PECR, FUT2, ADH1A, UGT2B7, HACL1, THEM4, CYP2C9, CYP4A11/CYP4Z2P, CYP3A5/ZSCAN25*), while the remaining were associated with fatty acids (saturated fatty acids: *MCM6*-pentadecanoic acid, *SEC14L6*-tricosanoic acid, and *SCARB1*-lignoceric acid; polyunsaturated fatty acid: *NTAN1/PDXDC1/NPIPA8*-dihomo-gamma-linolenic acid). The percentage of trait variance explained by top variants for these associations was low (percent variance range:

0.40%–1.26%). Conditional analyses only yielded an additional significant secondary signal for *CYP2C9* associated with a putative novel oxylipin (m/z = 365.2693, RT = 0.351) (Table S5). Three of these top variants, each associated with an oxylipin, are missense variants (*PECR*-rs9288513, *HACL1*-rs74637339) and one is a stop gained mutation (*FUT2*-rs601338).

Notably, three of these loci (*PECR*-rs9288513, MAF = 0.13), (*CYP2C9*- rs9332172, MAF = 0.17), and (*FUT2*-rs601338, MAF = 0.36) were all associated with the same oxylipin, a "putative novel oxylipin" (m/z = 365.27, RT = 0.351) that exhibited the highest heritability of all oxylipins (h_{SNP}^2 = 16.9% [95% CI: 10.9%–23.5%]). Additionally, this oxylipin was also associated with the pleiotropic *SLCO1B1* and *ACOT* loci. Together, these five lead variants explained 5.0% of trait variance (Table S2). This oxylipin was rather anomalous compared to many of the other oxylipins and fatty acids, the majority of which (37 of 40) were only significantly associated with one or two genetic loci.

Joint effects GWAS

Joint two-degree-of-freedom tests, which were conducted to increase the power to identify additional loci in which genetic variants exhibit both a main effect and interaction effect with waist circumference,[28,29] identified 35 fatty acids and oxylipins with at least one genome-wide significant locus ($p_{joint} < 6.94E{-}11$) (Table S12). The chromosome 13 *CARS2* locus, which was associated with a leukotriene (m/z = 480.25, RT = 0.351), was the only locus not previously identified in our main effect GWAS ($p_{main\ effects} = 0.16$). The *CARS2* top variant rs183091953 was significant among females (joint effects $p_{joint,\ females} = 2.00E{-}15$), but it did not pass the effective $N > 30$ filter in males (Table S12). Top variant rs183091953 was infrequent in HCHS/SOL participants (MAF = 0.002, effective $N = 30.5$), and is monomorphic in 1000 Genomes African, European, and South and East Asian reference continental ancestry groups.

Discussion

Summary

In this study, we performed one of the largest GWAS of fatty acids and oxylipins, which contribute to the initiation and resolution of inflammation. Our GWAS identified 19 genetic loci association with 40 oxylipins and fatty acids, including several associations driven by missense or stop gained variants. This work builds on prior GWAS of polyunsaturated fatty acids and a small number of oxylipins and illustrates the shared and distinct genetic architecture affecting fatty acid and oxylipin levels. These results provide insights into the genetic factors influencing circulating fatty acids and oxylipins, and motivate future multi-omics work to better characterize these metabolites and their roles in disease pathways.

Heritability

Previous studies have reported a wide range in heritability estimates (0%–~80%)[12,54,55] across a variety of metabolites, with significant differences often reported for different metabolite classes.[54] One previous study of serum oxylipins and polyunsaturated fatty acids in fasting samples from 138 participants from the University College Dublin twin study[56] reported heritability estimates up to 74% for oxylipins, which is much larger than our oxylipin heritability estimates. However, given that all heritability estimates are population specific, we know that distinct estimates may be driven by genetic and environmental factors, such as diet. For example, the highest heritability estimates in our sample were reported for polyunsaturated fatty acids that are far along the enzymatically regulated biosynthesis pathway from essential dietary fatty acids linoleic acid and alpha linolenic-acid (e.g., arachidonic acid, adrenic acid), whereas the heritability estimates of essential dietary fatty acids linoleic acid and alpha-linolenic acid were much lower. We did identify genome-wide significant associations for several multiple oxylipins and fatty acids that did not exhibit significant heritability. For these metabolites, their top associated variants all explained a very low proportion of variance, which likely explains why overall heritability was not significant despite these significant associations.

Comparison to previous findings

All of the variants we identified in our main effects GWAS, or high LD proxies, have been previously associated with fatty acid metabolites, phospholipids or other unannotated metabolites, as reported in the GWAS Catalog.[57] Furthermore, two recent studies in the Framingham and Atherosclerosis in Communities (ARIC) cohorts also identified many of the same loci, and several of the same top variants, associated with a variety of oxylipins and fatty acids as we report here, including *FADS, SLCO1B1, UGT2B7, PKD2L1, ACOT6, PECR, THEM4, ADH1A*, and multiple *CYP* loci.[58,59] This supports our results, though future work will be needed to better characterize the specific oxylipin subgroups that are influenced by each locus. Contrary to previous studies,[12,19] we did not identify associations between genes encoding lipoxygenase (LOX) enzymes (*ALOX5, ALOX12, ALOX12B, ALOXE3, ALOX15, ALOX15B*)[60] and oxylipins derived from these enzymatic reactions. Our inability to replicate these associations may be due to the composition of oxylipin species quantified in our samples; for example, there were relatively few HETE oxylipins. This finding may reflect heterogeneity in the composition of fatty acid and oxylipin profiles in our data due to diet and other factors. Similarly, we did not identify previously reported associations between the *ELOVL2* locus and eicosapentaenoic acid, docosapentaenoic acid, docosahexaenoic acid, and adrenic acid.[16,59]

Biological function and activity

Several of the associations we identified are consistent with known activities and pathways involved in fatty acid and

oxylipin biosynthesis.[8,14,18,61] At the polymorphic *FADS* locus involved in fatty acid biosynthesis,[18] we found evidence of colocalization for several oxylipins and polyunsaturated fatty acids, as well additional top variants that did not exhibit evidence of colocalization across fatty acids and oxylipins (e.g., for dihomo-gamma-linolenic acid). We also identified associations with four CYP 450 genetic loci, which encode proteins that are known to play roles in oxylipin synthesis and metabolism, as well as metabolism of drugs and other endogenous compounds.[61–63] For example, a putative novel oxylipin (m/z = 313.2379, RT = 0.355) was associated with *CYP4A11, CYP4Z2P* (top variant rs9332172)*;* and another (m/z = 345.2431, RT = 0.351) was associated with *CYP3A5, ZSCAN25* (top variant rs776746), suggesting they could be derived via CYP enzymatic reactions, or affected by CYP metabolism. Additionally, putative HpOTrE (m/z, = 309.207 RT = 0.339) and HpODE (m/z = 311.2231, RT = 0.343), which were moderately correlated, were both associated with *CYP4F2,* a gene contributing to a variety of activities, including inflammatory mediator regulation and the metabolism of drugs and endogenous compounds.[64] Top variant rs2108622, which exhibited evidence of colocalization for these two oxylipins, is a missense variant (p.V433M) that causes decreased activity of CYP4F2 due to its destabilizing protein effect[65] and has been previously associated with differential warfarin dosing.[64] Since HpOTrE and HpODE oxylipins are derived primarily through LOX enzymatic reactions on linoleic acid, not through CYP enzymatic reactions,[66,67] this suggests that their association with *CYP4F2* may reflect metabolism that may be affected by this decreased protein activity.

Other loci underlying the genetic architecture shared between multiple oxylipins and fatty acids are also involved in more downstream effects, such as small molecule transportation or signaling, that have important consequences for many endogenous compounds. This may explain why phenotypic correlations between the sets of metabolites associated with these pleiotropic loci were modest, despite shared genetic architecture, since the shared loci influence activities that affect a variety of distinct oxylipins rather than affecting a step along a vertical biosynthesis pathway, such as *FADS*, where stronger phenotypic correlation might be expected alongside shared genetic associations. For example, the *SLCO1B1* locus, which was associated with sixteen oxylipins (mean correlation of 0.39) that exhibited strong evidence of colocalization (many of which also shared additional secondary signals in exact conditional analyses), encodes the hepatocyte-expressed OATP1B1 protein, a membrane influx transporter.[51] OATP1B1 has both exogenous substrates, including statin drugs, and endogenous substrates like oxylipins, bilirubin, and conjugated steriods.[51,68] Our top variant rs4149056, is a missense variant encoding a loss of function change, p.V174A, that decreases OATP1B1 function. Consequently, this variant and others have been associated with statin-induced myopathy and other related drug interactions.[50,51] However, previous studies on the effect of *SLCO1B1* variants on endogenous substrate transportation have been limited to bilirubin and were largely inconclusive.[51,69–71] The associations we identified between *SLCO1B1* and oxylipins motivate the need for future experimental studies to determine whether rs4149056 and other variants also impact oxylipin transportation.

In addition to shared genetic architecture, we also identified 13 loci associated with only one metabolite, indicating that there were also distinct genetic effects influencing circulating fatty acid and oxylipin levels. Among these distinct effect loci, the average percent of trait variance explained by our top variants was 0.76%, suggesting that many of the metabolites associated with these loci may be more polygenic, with additional unidentified small effect variants. The biological activities of these distinct loci may offer insights into the potential identity of putative novel oxylipins. For example, in addition to associations with OATP1B1 transporter protein locus *SLCO1B1* and CYP locus *CYP2C9*, one putatively novel oxylipin (m/z = 365.2693, RT = 0.351) was also associated with loci implicated in fatty acid elongation (*PECR,* top variant rs9288513 encoding p.F297L change),[72,73] and hydrolysis of activated fatty acids into their non-esterified forms (*ACOT6,* top variant rs72719658)[74] suggesting that this oxylipin could be a long chain fatty acid compound or related metabolite, though more formal analyses beyond the scope of this analysis will be necessary to characterize putative novel oxylipin metabolites. Emerging methods that integrate genomics and metabolomics are showing promise for metabolite annotation,[75] offering novel means to improve annotation given the ubiquity of large-scale genomics. Interestingly, this oxylipin was also associated with *FUT2* variant rs601338, which encodes the stop gained mutation p.W143X that inactivates the FUT2 enzyme, leading to lack of secretion of blood antigens[76] and apparent resistance to certain infections, but increased risk of some autoimmune diseases.[77,78] Understanding how this mutation may impact oxylipins is an important future direction.

Ancestry-enriched variants

This *FUT2* variant, along with other variants at many of the loci we identified, is known to have varied allele frequencies across continental ancestry populations.[77] In this analysis, we identified two variants (rs28600890-*ADHA1* and rs183091953-*CARS2*) that are in fact monomorphic in at least one other 1000 Genomes continental ancestry reference population, but not in the admixed American HCHS/SOL sample, demonstrating how genetic analyses of ancestrally diverse populations can enable the identification of additional associations. *ADH1A* top variant rs28600890, which is monomorphic in European and South and East Asian 1000 Genomes reference continental ancestry populations, is in high LD (D′ = 1) with variant rs28864441, which was previously associated with unannotated metabolite levels in an African ancestry

population.[79] Furthermore, by extending our GWAS models to perform two-degree-of-freedom tests to detect both variant and variant-waist circumference-interaction effects on circulating oxylipins, we identified a rare variant specific to admixed American continental ancestry populations near the *CARS2* locus associated with a leukotriene (m/z = 480.25, RT = 0.351). Although this finding requires external replication, particularly considering the low frequency of the top variant, one recent study demonstrated an anti-inflammatory role for *CARS2* in macrophages and smooth muscle cells,[80] suggesting a biologically plausible link between *CARS2* and the leukotriene oxylipin.

Strengths and limitations

Utilizing a high-throughput and sensitive LC-MS method for comprehensively profiling fatty acids and oxylipins and conducted in a sample of over 11,000 participants, this study is one of the largest GWAS of oxylipins and related fatty acids to-date. Moreover, it was conducted in the ancestrally diverse HCHS/SOL cohort, which is important given that most genetic association and metabolomics studies have been historically conducted in European ancestry populations. Indeed, we identified two variants (rs28600890-*ADHA1* and rs183091953-*CARS2*) that are monomorphic in the 1000 Genomes European continental ancestry reference population, but not in admixed American reference populations, thus enabling identification of these signals in the HCHS/SOL cohort.

However, there are also several limitations to this study. First, formal replication of metabolite-genetic locus associations in independent populations is challenging, due to differences in measurement platforms and annotation of metabolites. In particular, we identified multiple significant associations with putative novel oxylipins, and therefore formal replication will not be possible until these can be more definitively annotated. Second, although circulating oxylipin concentrations can become elevated due to secretion or spillover from inflamed tissues,[81] the actions of oxylipins are likely highly localized and specific to tissue and cell types, which can be challenging to measure in non-experimental settings. Thus, our results may not capture genetic associations that underly tissue-specific oxylipin activity and profiles. However, they still provide insights into systemic oxylipin and fatty acid profiles and the pathways in which they operate.

Conclusions

Our study revealed shared and distinct genetic architecture underlying correlated and modest-to-moderately heritable fatty acids and bioactive oxylipins. The genetic architecture included loci involved in a variety of biological functions, including fatty acid and oxylipin synthesis, exogenous and endogenous substrate transport and metabolism, and inflammation regulation. These results provide insights into the genetic factors influencing circulating bioactive lipids, and motivate future multi-omics work to better characterize these compounds and elucidate their roles in disease pathways.

Data and code availability

Summary statistics for the main effects GWAS analyses are available on the GWAS Catalog (https://www.ebi.ac.uk/gwas/), study accession numbers: GCST90461825-GCST90461905. The data supporting the current study have not been deposited in a public repository because data are not public, but may be available from the corresponding author on request. The code can be requested from the corresponding author upon reasonable request.

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Author contributions

CGD, CLA, and HMH designed the study. CGD performed the statistical analysis. MJ, TL, and SC generated the oxylipin data. CGD and CLA wrote the manuscript with input from all authors. CGD and CLA had full access to the study data and take responsibility for the integrity of the data and accuracy of analyses. All authors have reviewed and approved the final manuscript.

Declaration of interests

Mohit Jain, Tao Long, and Nick Miller are employees and equity holders of Sapient Bioanalytics. None of the other authors had any financial or other conflicts of interest to disclose.

Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.xhgg.2024.100390.

Web resources

Github for heritability calculations: https://github.com/tamartsi/Heritability_CIs.
PubChem: https://pubchem.ncbi.nlm.nih.gov.
HMDB: https://hmdb.ca.
Cayman Chem: https://www.caymanchem.com.
Coloc: https://cran.r-project.org/web/packages/coloc/coloc.pdf.

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