A large-scale genome–lipid association map guides lipid identification

Vanessa Linke1, Katherine A. Overmyer2,3, Ian J. Miller3, Dain R. Brademan1, Paul D. Hutchins2, Edna A. Trujillo1, Thiru R. Reddy2,3, Jason D. Russell2, Emily M. Cushing4, Kathryn L. Schueler4, Donald S. Stapleton4, Mary E. Rabaglia4, Mark P. Keller4, Daniel M. Gatti5, Gregory R. Keele5, Duy Pham5, Karl W. Broman6, Gary A. Churchill5, Alan D. Attie4 and Joshua J. Coon5,6

Despite the crucial roles of lipids in metabolism, we are still at the early stages of comprehensively annotating lipid species and their genetic basis. Mass spectrometry-based discovery lipidomics offers the potential to globally survey lipids and their relative abundances in various biological samples. To discover the genetics of lipid features obtained through high-resolution liquid chromatography–tandem mass spectrometry, we analysed liver and plasma from 384 diversity outbred mice, and quantified 3,283 molecular features. These features were mapped to 5,622 lipid quantitative trait loci and compiled into a public web resource termed LipidGenie. The data are cross-referenced to the human genome and offer a bridge between genetic associations in humans and mice. Harnessing this resource, we used genome–lipid association data as an additional aid to identify a number of lipids, for example gangliosides through their association with B4galnt1, and found evidence for a group of sex-specific phosphatidylcholines through their shared locus. Finally, LipidGenie’s ability to query either mass or gene-centric terms suggests acyl-chain-specific functions for proteins of the ABHD family.
Fig. 1 | LC–MS/MS lipidomics and QTL mapping as ways to lipid identification. a, A modified MTBE lipid extraction was performed on plasma and liver from 64 FS and 384 DO mice. b–d, Lipid extracts were analysed by LC–MS/MS. Identifications were obtained through LipiDex on the basis of retention time window (b), exact mass (c), retention time window and tandem mass fragmentation (d). e, Quantitative values over large dynamic ranges for both identified and unidentified features were obtained. f, All lipidomic features (identified and unidentified) were then mapped onto the mouse genome via QTL mapping, revealing genomic position and FS allele effect pattern as results for each QTL. This additional information enabled identification of otherwise unidentified features. RT, retention time; HF/HS, high-fat/high-sucrose.
candidate genes for the lipid features in this study. Further, each of the eight inbred DO founder mouse strains (129, A/J, B6, CAST, non-obese diabetic (NOD), NZO, PWK, WSB) contributes to generate distinct allele effect patterns at each locus, thus providing an additional criterion for gene identification. Finally, careful control of external sources of variation such as diet and environmental conditions, allows the extraordinary phenotypic diversity of the DO to be directly attributed to genetic diversity. The DO population has already been used extensively to map clinical traits, transcripts, proteins, gut microbiota and bile acids, providing a wealth of existing data to integrate with global genome–lipid associations.

Here we describe discovery lipidomics analysis on a cohort of DO mice. In doing so, we use QTL position as an independent piece of information to guide lipid identification and apply it to define unidentified mass spectral features. We demonstrate the use of genome–lipid associations to assign identification or function in independent studies through a web-based resource, LipidGenie (http://lipidgenie.com/).

Results

QTL mapping connects lipids to their genetic regulators. To explore how global association of MS data to genomic coordinates could assist in lipid identification, we collected whole lipidome profiles of plasma and liver tissue from 64 FS and 384 DO mice by using high-resolution LC–MS/MS (Fig. 1a). Altogether, we performed 894 LC–MS/MS discovery lipidomics experiments from which we extracted approximately 4,500,000 tandem mass spectra (Fig. 1d). From the full scan MS data (Fig. 1c), we detected and quantified 19,636 molecular features: 12,429 in plasma and 7,207 in liver (Fig. 1b). Next, we applied the LipiDex algorithm to (1) match the tandem mass spectra to their respective features, (2) eliminate features derived from addition, dimerization, in-source fragmentation and so on, and (3) to assign molecular identities when possible (Fig. 1d). From the 3,283 distinct molecular features that remained, we identified 594 lipids (from 1,721 features) in plasma and 584 lipids (from 1,562 features) in liver (see Lipidomics data analysis in the Methods for details). This discovery approach allows for broad, untargeted lipidome coverage. However, we note that some lipid classes, including PS, LysoPS, PA, LysoPA, LysoPG and cholesterol, are either partially or wholly, missed by the current method. For instance, reversed-phase–electrospray ionization (ESI)–tandem MS poorly retains phosphatic acid (PA) and phosphatidylserine (PS) species. Further, cholestersols and other sterols have poor ESI efficiencies, Extended Data Fig. 2a,b and Table 1 provide an overview of the identified lipids that span roughly 30 lipid subclasses from five of the main classes: fatty acyls, glycerolipids, phospholipids, sphingolipids and sterol lipids. For ~70% of these identifications, we find MS/MS evidence to detail fatty acid composition; otherwise, we report sum composition.

Extended Data Fig. 1a,b presents a bird’s-eye view of these plasma and liver lipoproteins. Here each distinct molecular feature is plotted as a function of its m/z and chromatographic retention time. Identified lipids are coloured by class; we note members of individual lipid classes group well, adding confidence to their identification. Triglycerides, for example, as hydrophobic lipids with three fatty acids can be found at high m/z and late chromatographic retention. From this perspective, we observe that the unidentified molecular features, two-thirds of all detected species, are either clustered around identified lipid classes or exist on m/z and retention islands. We conclude that these data can be further interrogated to (1) expand existing lipid class coverage and (2) reveal the presence of additional lipid classes.

Next, we extracted quantitative information from all detected molecular features across all 384 animals, creating a molecular trait for each feature. Figure 1e displays the quantitative values of two examples of individual molecular traits from plasma, one identified as a sphingolipid and one unidentified. Plasma HexCer[N] d18:1_20:0 has a relative abundance dynamic range of ~15-fold across all 384 animals. For comparison, we plot the abundance of a molecular feature with a mass of 1,252.8028 Da. Here we see an even greater dynamic range of ~75-fold, however, the feature was unidentified using our traditional data processing. Correlation to a candidate gene region ultimately led to the identification of the feature. To correlate these MS-derived lipid quantitative phenotypes (vide supra) with genomic variation we performed quantitative trait locus (QTL) mapping using R/qtl (ref. 27).

Figure 2a displays a hierarchically clustered heatmap of these quantitative results for all measured molecular traits (1,721 and 1,562 for plasma and liver, respectively) across all 384 animals. Notably, we observe considerable clustering by lipid class, even across tissue type (y axis). We detected 3,348 plasma lipid QTL for 1,405 of the 1,721 (81.6%) traits (logarithm of odds (LOD) score $> 6$). 1,351 of these were from identified lipids, while 1,997 were from unidentified features. Similarly, in liver, we detected 2,269 lipid QTL for 1,190 of the 1,562 (76.2%) traits, of which 927 were from identified lipids while 1,342 were from unidentified features. Fig. 2b and Extended Data Fig. 2c,d present the genetic correlations for this entire collection of substantial QTL extracted in a Manhattan plot. We note that the unidentified molecular traits cluster among the various identified lipid classes, which provides further evidence that these features are of biological origin and amenable for further interrogation. Second, the unidentified features occupy additional distinct loci, implicating previously unidentified lipid classes.

QTL map recapitulates APOA2 biology and informs cholesteryl ester lipid identifications. Several genetic loci are strongly associated with lipids and appear as hotspots: locations on the genome where multiple lipid QTL comap (Fig. 2b and Extended Data Fig. 2b). To better explore these regions, we asked whether these comapping lipid QTL shared a common genetic relationship to segregating alleles at the locus. One advantage of the DO mice is that shared FS allele effect patterns can be indicative of a common genetic regulator. Thus, we define a lipid QTL hotspot as multiple lipid QTL comap (±2Mbp) with a shared FS allele effect pattern. We identified a number of hotspots; many of these are detailed in Supplementary Table 1 with their respective lipid class and probable candidate gene drivers. To garner additional support for FS specific genetic effects on lipid abundance, we profiled plasma and liver lipids for each of the FSs (four males, four females; see Supplementary Tables 10 and 11).

Figure 3a highlights a lipid QTL hotspot on chromosome 1:171Mbp. Here, 255 lipid traits, all from plasma, colocalize with a shared allele effect pattern of upregulation associated with alleles derived from the FS 129 (Extended Data Fig. 3a). The lipid with the highest LOD at this locus was a cholesteryl ester (CE 18:2), which was also elevated in FS 129 plasma (Extended Data Fig. 3b). At 171Mbp on chromosome 1, strain 129 possesses a missense single nucleotide polymorphism (SNP) in Apoa2 gene (rs8258226), resulting in a 61A Ala=Val substitution in the protein apolipoprotein-II (ref. 40). A previous DO study identified APOA2 protein and mRNA expression QTL in liver tissue but these displayed different allele effects than plasma lipid QTL, suggesting that the causal variants that modulate their respective levels differ (Extended Data Fig. 3c)41. Notably, APOA2 protein is a chief component of high-density lipoprotein (HDL) particles in plasma, corroborated by human HDL traits mapping to APOA2 in GWAS, and is considered a principal genetic regulator of plasma HDL levels in mice. The other chief components of HDL particles are phospholipids (35–50%) and cholesteryl esters (30–40%) (Fig. 3b)42. Consistent with this composition, seven subtypes of phospholipid and various cholesteryl esters map to the Apoa2 locus (Fig. 3c). Sphingolipids, a minor component of HDL particles, map in four different
To test whether a shared QTL would enable identification of additional lipids, we plotted the 255 lipid traits that map to the Apoa2 locus as a function of chromatographic retention time, mass and identification status (Fig. 3d). A cluster of unidentified lipid features shared retention time with cholesteryl esters, a class of lipids that are often devoid of informative fragments. All cholesteryl esters showed their main QTL at the Apoa2 locus (Fig. 3e) providing greater confidence in their identification. The shared genetic regulation further allowed us to predict a cholesteryl ester identity for the cluster of unidentified comapping features. Examination of their total masses and tandem mass spectra supported the annotation of five additional cholesteryl esters (Fig. 3f), while another 18 lipid features’ m/z and retention time were consistent with technical artefacts of cholesteryl esters: 11 heterodimers, four cholesterol adducts and three in-source fragments.

QTL map provides an orthogonal tool for lipid identification. On chromosome 10, at 127 Mbp we observed a notable lipid QTL hotspot. At this site, over 25 plasma and liver lipid features mapped with the highest overall importance (Fig. 4a). These features also shared a common allele dependence, that is, NOD-driven and split between NOD high versus low effect (Fig. 4b). None of these lipid features were identified following our conventional data analysis strategy, which leverages retention time, mass and tandem mass spectra. The features were observed in two distinct clusters on the basis of m/z and retention time, suggesting they could derive from two distinct lipid classes (Fig. 4c). Given that these unidentified features (1) appeared as two defined lipid classes and (2) subclasses to this Apoa2 locus. We conclude that this hotspot illuminates the molecular composition of HDL particles in mice, while also linking an additional 130 unidentified lipid features to this locus.

Table 1 | Breakdown of lipid identifications in plasma and liver samples by one of 31 classes

| Lipid category | Lipid class | Abbreviation(s) | Count | Percentage of total | Molecular level | Percentage of IDd | Count | Percentage of total | Molecular level | Percentage of IDd |
|----------------|-------------|----------------|-------|---------------------|----------------|-----------------|-------|---------------------|----------------|-----------------|
| Fatty acyl      | Acylcarnitine | AC             | 2     | 0.3                 | 2              | 100.0           | 6     | 1.0                 | 6              | 100.0           |
|                | Fatty acid*  | FA             | 27    | 4.3                 | 27             | 100.0           | 31    | 5.0                 | 31             | 100.0           |
| Glycolipid     | Diglyceride  | DG, alkyl-DG   | 2     | 0.3                 | 2              | 100.0           | 17    | 2.7                 | 15             | 88.2            |
|                | Triglyceride | TG, alkyl-TG, alkyl-TG | 208 | 32.8               | 163            | 78.4            | 168   | 27.0                | 96             | 57.1            |
| Phospholipid   | Cardiolipin  | CL             | 129   | 20.3               | 69             | 53.5            | 88    | 14.1                | 62             | 70.5            |
|                | Lyso-phosphocholine | LysoPC       | 27    | 4.3                 | 27             | 100.0           | 18    | 2.9                 | 18             | 100.0           |
|                | Lyso-phosphethanolamine | Lyso-PE    | 5     | 0.8                 | 5              | 100.0           | 10    | 1.6                 | 10             | 100.0           |
|                | Lyso-phosphoinositol | Lyso-PI    | 1     | 0.2                 | 1              | 100.0           | 3     | 0.5                 | 3              | 100.0           |
|                | Phosphocholine | PC            | 24    | 3.8                 | 22             | 91.7            | 80    | 12.8                | 57             | 71.3            |
|                | Phosphoethanolamine | PE, PE-NMe2 | 2     | 0.3                 | 2              | 100.0           | 26    | 4.2                 | 26             | 100.0           |
|                | Phosphoglycerol | PG           | 23    | 3.6                 | 19             | 82.6            | 23    | 3.7                 | 21             | 91.3            |
|                | Phosphoinositol | PI            | 76    | 12.0               | 49             | 64.5            | 46    | 7.4                 | 33             | 71.7            |
| Plasmalogen     | Plasmalanyl-Pe, Plasmenyl-Pe, Plasmanylyl-Pe, Plasmenyl-Pc | 40 | 6.3               | 34             | 85.0            | 58    | 9.3                 | 39             | 67.2            |
| Sphingolipid    | Ganglioside* | GM2/GM3        | 13    | 2.1                 | 7              | 53.8            | 8     | 1.3                 | 6              | 75.0            |
|                | Sphingomyelin | SM            | 49    | 7.7                 | 0              | 0.0             | 29    | 4.7                 | 0              | 0               |
|                | Sphinogosine | SP             | 0     | 0                  | 0              | 100.0           | 0     | 0                  | 0              | 0               |
| Sterol lipid    | Cholesteryl ester | CE          | 6     | 0.9                 | 6              | 100.0           | 2     | 0.3%                | 2              | 100.0           |
| Plasmalogen     | Plasmalanyl-Pe, Plasmenyl-Pe, Plasmanylyl-Pe, Plasmenyl-Pc | 76 | 12.0               | 49             | 64.5            | 46    | 7.4                 | 33             | 71.7            |

*Molecular level’ refers to lipids identified with individual fatty acid rather than as a sum composition. ‘Hand-identified.

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Articles were high scoring at a genetic locus with opposite allele effects, we reasoned that identification of the causal gene may enable their identification.

The genetic effects of SNPs and other genomic variants can influence lipid abundance. For example, SNPs in coding regions can affect protein product function. In the extreme, a missense variant

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**Fig. 2** | Large-scale lipid quantitative profiling and subsequent QTL mapping reveals hotspots of associated lipids. **a**, In plasma, we quantified 1,721 lipidomic features, 621 of which were identified, and in liver, we quantified 1,562 lipidomic features, 615 of which were identified. Hierarchical clustering of all 3,283 lipidomic features’ intensities by the 384 DO mice resulted in distinct clustering by lipid class, notably across tissue type. **b**, When mapped onto the mouse genome, 1,405 plasma and 1,190 liver features showed at least one QTL with an LOD > 6 as displayed in a Manhattan plot (n = 3,353 + 2,269 = 5,622 total QTL). A number of lipid hotspots are shared by identified lipids and unidentified features (for example, at *Apoa2*), while others only appear among the unidentified features (for example, at *B4galnt1*). Chr, chromosome.
Fig. 3 | Comapping of lipids at the Apoa2 locus facilitated identification of additional cholesteryl esters. a, One lipid hotspot on chromosome 1 at 171 Mbp is shared by 255 plasma lipid features comapping with a common 129 high allele effect (Extended Data Fig. 3a). b, The candidate gene at this locus is Apoa2, which encodes apolipoprotein-II, which is carried on HDL cholesterol particles along with c, a variety of lipid classes, mostly phospho- and sphingolipids, which mapped to the locus. d, When plotting all 255 Apoa2-specific lipid features in the m/z–retention time (RT) plane, a group of unidentified features sharing the RT region with cholesteryl esters (CEs) stood out. e, Notably, all six CEs show their primary QTL at this locus, as visible from their individual LOD plots. f, Subsets of the unidentified features could subsequently be identified as CE-related features, including heterodimers, cholesterol adducts and in-source fragments. FA (fatty acid), PE (phosphatidylethanolamine), PI (phosphatidylinositol), RKMD (referenced Kendrick mass defect)48.
in proteins involved in lipid metabolism could probably affect lipid abundance. SNPs in non-coding regions, such as promoters and enhancers, can alter gene expression. To identify candidate SNPs, we analysed the SNPs associated with the lipids by identifying those with the FS SNP database at each QTL (R/qtl2, scan1snps(10)) and subsequently causing missense, frameshift, stop lost/gained, incomplete terminal codons, in-frame deletions/insertions, altering 3’ or 5’ untranslated region (UTR) sequences, splice acceptor/donor/region, predicated to cause nonsense-mediated decay, initiator codon or mature miRNA variants (according to the Sequence Ontology consortium)⁴⁹. At the chromosome 10 hotspot, we identified several candidate genes with potentially causal mutations (Fig. 4d). We included in our analysis, but did not focus on genes with synonymous, stop retained, upstream/downstream, intergenic, intron and non-coding transcript (exon) variants (which represent ~97% of all SNPs in the database).

In cases of altered gene expression, we further narrowed down the list of candidates by directly assessing transcriptomics data. While we did not profile hepatic gene expression in the DO cohort used for lipid QTL analysis, we surveyed a recently published hepatic QTL dataset to match allele effects of mRNA expression and protein QTL that are within the location of the candidate gene (cis-eQTL and pQTL, respectively)⁵⁰. We asked if any transcripts or proteins presented a similar NOD-driven allele effect at the lipid locus on chromosome 10. Of the protein coding genes within ±2 Mb of the lipid QTL, 55 showed a cis-eQTL (Supplementary Table 2). However, the only cis-eQTL that was strongly and uniquely driven by NOD alleles was B4galnt1 (Extended Data Fig. 4a). Furthermore, 16 cis-pQTL were identified for genes within this region, including B4GALNT1 (Supplementary Table 3). Similarly to the cis-eQTL, the only pQTL that showed a NOD-driven allele effect pattern was for B4GALNT1 (Extended Data Fig. 4b). Consequently, the 3’ UTR variant in B4galnt1 SNP rs13462597 was our strongest candidate as the genetic regulation of hepatic B4galnt1 transcript and protein expression matches that of the unidentified lipids.

B4galnt1 encodes β-1,4 N-acetylgalactosaminyltransferase 1, an enzyme that catalyses the conversion of GM3 to GM2 gangliosides⁵¹. With this candidate gene in mind, we investigated whether the unidentified lipids could be classified as gangliosides. Their precursor m/z and tandem mass spectra were consistent with monosialic gangliosides, which we further confirmed by comparison with a GM3 ganglioside standard (Fig. 5c). In total, we confidently identified 26 lipid features as six unique GM2 and seven unique GM3 species (Supplementary Table 4). In agreement with a NOD-driven effect, NOD mice have higher abundance of GM3 gangliosides in the pancreas⁵² and we confirmed NOD had higher abundance of GM3 in plasma in an independent lipidomic analysis of FS mice (Extended Data Fig. 4c).

By identifying the features mapping to chromosome 10:127 Mb as gangliosides, we recognized that ganglioside abundances, such as the levels of most lipid species, were polygenic, that is, regulated by multiple loci (Fig. 4f). From the 26 identified ganglioside features we gain a total of 62 QTL annotations, describing more than 15 unique loci (at least two ganglioside features with LOD > 6.0) on ten chromosomes (Supplementary Table 5). These newly annotated ganglioside QTL mapped to candidate genes of the ganglioside pathway (Sgms1, ref. 53; B3galt4; St3gal2; Cmah), even more distant regulators of ganglioside metabolism (Slc9a6, ref. 54; Cog2, ref. 55; Trep5, ref. 56; Cdh13) and regions of the genome with yet undescribed ganglioside regulation (Fig. 4g).

LipidGenie identifies candidate genetic regulators for lipid features. To make these genome–lipid associations accessible to the community we created a web-based resource, LipidGenie (http://lipidgenie.com). With LipidGenie, lipid features can be searched by m/z, lipid identifier or lipid class. The search returns QTL of the matching features and allows the user to explore the genetic region, FS allele effects and associated SNPs. In addition, with LipidGenie individual genes or gene regions can be queried for lipid associations.

To validate LipidGenie we explored sex-associated lipid features that were observed within the B6 FS. In this study, we quantified 2,558 lipid features in B6 plasma and found 254 features that showed notably different levels by sex (Fig. 5a). As is common in LC–MS lipidomics, most of these sex-specific features were unidentified after the database search (n = 197). Using LipidGenie’s m/z search parameter and a 10 ppm m/z window, we found notable genome–lipid associations for 127 of the sex-specific features, of which 79 were unidentified. A group of six unidentified lipids mapped to the same genetic locus on chromosome 6 at 91 Mb (Fig. 5b and Supplementary Table 6); all had similar allele effect patterns (Extended Data Fig. 5a) and were elevated in males (Fig. 5a and Extended Data Fig. 5b). At the locus, a total of 12 out of 21 conmaping features shared a lipid class-like behaviour, that is, clustered in m/z–RT space (Fig. 5c). To further characterize these lipids, we collected additional tandem mass spectra in both positive and negative mode (Fig. 5d–g).

The spectra showed shared fragmentation patterns consistent with a phosphatidylcholine (PC) class identity. One fatty acid seemed to be either fatty acid 22:6 (Fig. 5e) or fatty acid 16:0, but only MS3 spectra showed the presence of a second acyl-chain expected for PCs (Fig. 5f, g). These features also shared an m/z 522 fragment that matched the formula of LysoPC 19:0, C₅₀H₇₉NO₇P⁻. We next used the LipidGenie associations to generate hypotheses about the nature of this lipid class. At chromosome 6 Mb, we found SNPs with matching allele effects in several genes including Tmxrd3, Vmnt4, Uroc1, Aldh11l1, Slc41a3, Grip2 and Tnh (Fig. 5b). One possible candidate on chromosome 6 is Vmnt4, encoding for vomeronasal receptors, the organs that sense pheromones. This gene explained the observed sex difference and also points to PC estolides as a potential class identity. Estolides are lipids containing fatty acid esters of hydroxy-fatty acids (FAHFAs). Consistent with the observed 16:0 or 22:6 fragments in MS2 spectra of the unidentified lipids, 16:0 and 22:6 can be esterified to hydroxy-fatty acids to form FAHFAs⁵⁷. This hypothesis is further supported by accounts of FAHFAs as pheromones in spiders and triglyceride estolides in mammalian scent glands⁵⁸. The potential estolide identity is intriguing, but definitive identification will require follow-up studies. Further evidence is probably contained in the genetic associations. Similar to our earlier example with gangliosides, we observed conmaping of these 12 lipids at other loci (for example, Chr 1, at

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**Fig. 4** Lipid features mapping to B4galnt1 lead to identification of GM2 and GM3 gangliosides. **a,b** A hotspot of solely unidentified features with exceptionally strong correlation was composed of 11 liver and 15 plasma features mapping to chromosome 10:127 Mb (a) with a similar NOD-driver allele pattern (b) (two main clusters from hierarchical clustering, row-scaled, Euclidean cutoff of h = 2.5). **a-c** Two groups of lipid features (circles versus triangles) emerged as distinct in strength of LOD (a), directionality of allele effect (b) and m/z space (c). **d,e** The candidate gene B4galnt1 pointed us to the putative identities of GM3 (circles) and GM2 (triangles) gangliosides (d), which were confirmed by spectral matching with a human GM3 standard (e). **f** Secondary QTL for these gangliosides, as exemplary shown for GM2 d18:1_22:0, mapped to eight additional candidate genes within 4 Mb of the 15 total ganglioside hotspots that were previously linked to ganglioside metabolism. **g** The various candidate genes influencing GM3 and GM2 levels span well-known enzymes (for example, B3galt4) but also include indirect affecters including Cog2 and Slc9a6. NOD (mouse strain NOD/ShiLtJ), Glc (glucose), GalNAc (N-acetylgalactosamine), Gal (galactose), NGNA (N-glycolyneraminic acid), NANA (N-acetylneuraminic acid).
84 Mbp, Chr 12 at 84 Mbp), thereby offering potential pathway information (Supplementary Table 7) and highlighting the power of genome–lipid associations obtained with LipidGenie.

We next explored whether LipidGenie would also offer insights when querying for identified lipid features. Recently, Parker et al. found an association between LysoPC 14:0 and chromosome 5.
Fig. 5 | Web resource LipidGenie guides exploration of genome–lipid connections. a, We quantified 2,558 features in B6 plasma (n = 4 for each sex). 254 features were sex-specific (fold change (FC) > 1.0, P < 0.05, non-paired, two-sided Student’s t-test). Precursor m/z (±10 ppm) matching to our DO database provided genetic information for one-third of the otherwise unidentified features. b, Six male-specific unidentified features (red) share a QTL on Chr 6:91 Mbp with a common A/J down effect (Extended Data Fig. 5a). c, The features further clustered in m/z-RT space. d-g. Targeted fragmentation spectra (exemplary spectra for two species ([M+H]+ m/z 1,156 (d-f) and 1,158 (g)) in positive (MS2) (d) and negative (MS2 (d,e) and MS3 (f,g)) mode) exhibited signals consistent with a lipid class built of a PC headgroup and three FAs. h-k, The DO database further confirmed LysoPC 14:0 mapping to Abhd1 (ref. 59); (h) with an enrichment of fatty acid 14:0 (j) containing lipids (k). We compare Hepa1-6 overexpressing ABHD1 and ABHD3 versus a control overexpressing GFP (n = 12 for each, four biological x three technical replicates, boxplots are defined with first and third quartiles for lower and upper hinges, 1.5x interquartile range for the length of the whiskers, centre line at median). The boxplots show absolute FC of each mutant over GFP by lipid class; the dashed line is at FC of 0.4. I, The lowest (negative) FC for both is observed for LysoPC 14:0; isomers are summed. m, All 14:0-containing PCs exhibit a negative FC for ABHD1 and ABHD3 mutants consistently, while 18:0-containing species are showing opposing positive FC. Plotted are sum-normalized, log_{10}-transformed FC means with error bars representing 95% confidence interval, significance indicated by * P < 0.05, ** P < 0.01, *** P < 0.001 of non-paired two-sided Student’s t-test, equal variance, n = 12 for each details, in-source data. F/M (female-to-male).
at 31 Mbp using multi-omic QTL mapping of the hybrid mouse diversity panel\(^\text{49}\). From these data, they postulated that the protein encoded by candidate gene Abhd1 (alpha beta hydrolase domain containing 1) regulates plasma levels of LysoPCs. Of note, ABHD1 has no annotated function. LipidGenie’s lipid search provides a direct means to test this putative functional annotation of ABHD1.

LipidGenie confirmed that plasma LysoPC 14:0 has a strong QTL at the abhd1 locus (Fig. 5h), and further found the B6 and NZO high-allele effect consistent with the 3′ UTR variant rs29681817 (Extended Data Fig. 5c). This observation is further supported by an independent measure of the FS mice and a hepatic cis-eQTL in Abhd1 with matching opposite allele effects (Extended Data Fig. 5d, e). To connect the function of ABHD1 protein to LysoPCs, we asked whether other LysoPCs (n = 45) mapped to this gene region. However, we did not find general mapping of LysoPCs to this locus (Fig. 5i), but instead found other lipids comapping on chromosome 5, at 31 Mbp, including PC 14:0_16:0, PE 14:0_20:4, PE 14:0_22:6, PC 28:0, PC 30:0 and PC 30:1 (Fig. 5h). These fatty acid signatures suggest a myristic acid (14:0) specific association. Given the high degree of lipid structural resolution contained within LipidGenie, we demonstrate that 14:0-containing lipids (n = 30) have an enriched hotspot at the Abhd1 locus (Fig. 5j). With these data we propose that ABHD1 is a phospholipase for myristic acid containing phospholipids, consistent with the function of a related and highly homologous gene, abhd3 (refs. 50\textsuperscript{,}52\textsuperscript{,}53). Phospholipids containing 14:0 have also been mapped to ABHD3 in human GWAS\(^\text{54}\). To validate this hypothesis, we overexpressed ABHD1 and ABHD3 in Hepa-1-6 cells (Extended Data Fig. 6a,b) and measured their lipids with respect to cells overexpressing green fluorescent protein (GFP) as control (Supplementary Table 12). Hierarchical clustering of the top 49 features showed two clusters, one with increased levels in the mutants over control and the other one decreased (Extended Data Fig. 6c). We noticed most of the identified lipids among the most substantially different features, and when plotting the average fold change by lipid class, LysoPC and PC phospholipids stood out (Fig. 5k). On closer examination, we were able to confirm the predicted fatty acid dependency for both LysoPC and PC lipids, particularly prominent in 14:0-containing phospholipids (Fig. 5l,m). While ABHD1 and ABHD3 mutants exhibited largely similar lipidomic profiles, differences as in PC 16:1_20:4, which was decreased only in the Abhd3 mutant, may also point to differential functions. The 14:0 specificity could be relevant to human health as plasma LysoPC 14:0 is a predictor of diabetes risk in humans. Finally, our proposed function might provide a clue to understanding why ABHD1 is associated with oxidative stress, a prominent hallmark of metabolic diseases\(^\text{51}\text{,}52\text{,}54\).

Having documented the diverse use of LipidGenie for lipid queries, we finally tested its use for gene-based queries. ABHD2, another member of the alpha beta hydrolase domain protein family, acts on arachidonoylglycerol, among other substrates\(^\text{55}\text{,}56\). A LipidGenie query of Abhd2 does indeed provide evidence for this polyunsaturated fatty acid pathway specificity. Specifically, within 2 Mbp of Abhd2, LipidGenie returned ten liver phospholipids. Eight of these lipids shared an allele effect pattern, and contained polyunsaturated fatty acids; that is, 18:2, 18:3, 20:3, 20:4 and 22:6 (Extended Data Fig. 5f,g). Further, ABHD2 showed matching opposite WSF and CAST effects in both liver cis-eQTL and pQTL (Extended Data Fig. 5h,i)\(^\text{47}\).

**Discussion**

Discovery lipidomics currently relies on measurement of various chemical properties for lipid identification. These properties are most often hydrophobicity, mass and fragmentation pattern. Unfortunately, application of only these strategies to complex mammalian lipid mixtures results in many unidentified lipid features. Here we investigated the power of genome–lipid associations to facilitate lipid identification.

To construct a large-scale map of genome–lipid associations, we performed QTL analysis for over 5,000 plasma and liver lipid QTLs, of which over 60% stem from unidentified spectral features. To our knowledge, this QTL map is the broadest in scope and depth of lipids analysed and QTL identified in mice\(^\text{57}\text{,}58\text{,}64\). With these data, we first tested our hypothesis by analysing one of several QTL hotspots, the Apos2 locus. The identified lipids mapping to this locus belonged to 11 different classes and, together with APOA2, constitute the known components of HDL particles. With this association, 23 unidentified lipid features could be classified as cholesterol esters and related features.

To further test the concept, we selected a second hotspot containing only unidentified lipid features (chromosome 10:127 Mbp). Genetic mapping to B4galnt1 enabled their identification as GM3 and GM2 gangliosides. In fact, the identification allowed for a comprehensive investigation of their complex polygenic regulation. We identified a total of eight candidate genes that probably contribute different functions in the pathway, including three (Slc9a6, Cog2, Trpc5) that exert indirect effects on ganglioside biosynthetic enzymes.

Having confirmed the value of genome–lipid associations for lipid mass spectral data annotation, we built an interactive, queryable resource, LipidGenie. Using the lipid query function, we demonstrated LipidGenie’s ability to facilitate lipid identification and in one instance revealed a potentially new subclass of PC lipids (PC estolides). Beyond assisting lipid identification, LipidGenie can provide evidence for gene function, and when queried for either lipid or gene identity, LipidGenie revealed acyl-chain specificity for ABHD1 and ABHD2, respectively. We confirmed the putative phospholipase function of ABHD1 in cells overexpressing the mouse protein while comparing to ABHD3.

We envision the genome–lipid associations contained within LipidGenie to be a valuable resource for researchers across multiple fields. We anticipate it will be immediately useful for directed analysis of key unidentified features in exploratory lipidomics analyses and lead to recovery of more data for biological studies. A limitation of the approach is that lipid identification remains a manual process and this tool does not remove the requirement for expert knowledge and care in spectral interpretation for its use. With all this said, we hope it will garner excitement for potentially new genetic regulation of lipid metabolism. Finally, through integration with other large data resources, for example, protein–protein interactions, pathway tools, tissue-specific QTL, GWAS data and so on, these genome–lipid associations will allow more global integration of lipid data into current knowledge bases. Especially the integration with human loci will allow for cross-validation to provide information on human health and disease\(^\text{60}\text{,}70\).

**Methods**

**Animal husbandry and sample collection.** All experiments involving mice were preapproved by an AAALAC-accredited Institutional Animal Care and Use Committee of the College of Agricultural Life Sciences (CALS) at the University of Wisconsin–Madison. The CALS Animal Care and Use Protocol number associated with the study is A005821, A.D. Attie, Principal Investigator. Equal numbers of male and female DO mice and the eight FSs (C57BL/6J (B6), A/J, 129S1/SvImJ (129), NOD/ShiLtJ (NOD), NZO/HILtJ (NZO), PWK/PhJ (PWK), WSB/EiJ (WSB) and CAST/EiJ (CAST)) were all obtained from the Jackson Laboratories and have been previously described\(^\text{13\text{,}14\text{,}23\text{,}47}\). Briefly, all mice were housed within the vivarium at the Biochemistry Department, University of Wisconsin–Madison, and maintained on a Western-style high-fat/high-sucrose diet (44.6% kcalories of fat, 34% carbohydrate and 17.3% protein) from Envigo Teklad (TD 08811) for 16 weeks. All mice were maintained in a temperature and humidity-controlled room on a 12 h light/dark cycle (lights on at 6:00 and off at 18:00), and provided water ad libitum. At ~22 weeks of age, mice were killed following a 4-h fast. Plasma and liver were collected from each mouse and flash frozen in liquid nitrogen. One sample from each tissue per mouse was used for lipidomic analyses.

**Mouse genotyping and haplotype reconstruction.** We collected tail biopsies for DNA extraction\(^\text{71}\) at 4–6 weeks of age when animals arrived at the University of Wisconsin–Madison, and performed Affymetrix Mouse 430 2.0 GeneChip hybridization and analysis as described. All mice were genotyped and haplotyped at the Biochemistry Department, University of Wisconsin–Madison.

**Data analysis and statistics.** All statistical analyses were performed using R version 3.6.1 \((\text{https://www.r-project.org/})\). Data were preprocessed using the R package DistillR (Hoffman et al., 2017) to remove negative peaks and to apply a distinct positive scaling factor for each lipid and each tissue per mouse. Lipid features were then normalized to the mean intensity of the m/z 746.23 (18:2_20:4) peak for each mouse. Linear mixed effect models with fixed and random effects were fit using the lmer function in the R package lme4 (Bates et al., 2015) to identify significant associations of each QTL with each lipid. To account for multiple testing, per locus false discovery rate (FDR) correction was applied using the R package qvalue ( Storey and Tibshirani, 2003).

**Figure and table generation.** All figures and tables were generated using Adobe Illustrator and Microsoft Excel, respectively.
Wisconsin and were assigned to single-housed pens. We shipped DNA to Neogen for genotyping using the Mouse Universal Genotyping Array (GigaMUGA, 143,259 markers). Genotype cells were subject to quality control as described in Broman et al. Genotypes were used to reconstruct the eight-founder haplotype mosaic of each DO mouse using the hidden Markov model in the R/qtl2 software package. The haplotype reconstruction uses information at each genetic markers and its neighbours to assign an eight-state haplotype probability that accounts for both heterozygosity and uncertainty in haplotype assignments. We interpolated these eight haplotype probabilities onto an evenly spaced grid of 69,005 pseudo-markers for mapping analysis. Sample mix-ups (one pair of samples) were resolved using islet gene expression data as described in Keller et al.

Plasmids and cell culture expression. Mouse Abhd1 (CMV6 promoter, Myc-DDK-tagged, MR206471) and mouse Abhd3 (CMV6 promoter, Myc-DDK-tagged, MR206458) plasmids were obtained from Origene. The Myc-DDK-tagged, MR206458) plasmids were obtained from J. Simcox. All plasmids were transformed into Escherichia coli (ThermoFisher Scientific, 18258019). Plasmids were maxiprepared according to the manufacturer’s instructions (Qiagen, 12662). Then 5 x 10⁶ Hepa-1 cells (ATCC CRL-1830) were seeded in six-well plates with DMEM (ThermoFisher Scientific, 12100061). After 16h, cells were reconditioned with fresh medium for 2 h. Cells were transfected in triplicate with Lipofectamine2000 (ThermoFisher Scientific, 11668819) according to the manufacturer’s instructions. Transfection efficiency was confirmed by visualizing GFP. After 24h, medium was replaced. Then, 48h after transfection, cells were washed in cold 1x PBS and scraped to be released from the plate. Released cells were pelleted by centrifugation and snap-frozen in liquid nitrogen. The frozen cell pellets were stored at −80°C until lysis. Hepa-1 cells were a gift from J. Simcox.

For western blots, cell pellets were lysed in 2x SDS–PAGE loading buffer and boiled at 95 °C for 5 min. Samples were run on a 10% SDS–PAGE gel for 1.5 h at 120 V. standard is Precision Plus Dual Color Protein Standards (Bio-Rad, 1610394). Standard was run by Tune software v.2.9.3.2948 (Thermo Scientific) by a HESI II heated ESI source kept at 300 °C (Thermo Scientific). The inlet capillary was kept at 300 °C, sheath gas was set to 10 units, auxiliary gas to 10 units, and the spray voltage was set to 4,000 V(+) and 3,500 V(−), respectively. The MS was operated in polarity switching dd-MS2 mode acquiring positive and negative mode MS1 and MS2 spectra (Top2) during the same separation. MS acquisition parameters were 17,500 resolving power, 1 x 10⁵ automatic gain control (AGC) target for MS1 and 1 x 10⁴ AGC target for MS2 spectra. The MS was operated in polarity switching dd-MS2 mode acquiring positive and negative mode MS1 and MS2 spectra (Top2 for positive, Top3 for negative mode) during the same separation. MS acquisition parameters were 60,000 resolution and 3 x 10⁴ AGC target for MS1 and 15,000 resolution and 5 x 10⁴ AGC target for MS2 scans, 100-μs MS1 and 35-μs MS2 ion accumulation time, 200- to 2,000-Th MS2 scan range, 1-Th isolation width for fragmentation, stepped HCD collision dissociation (HCD) collision energy (20, 30, 40 units), 1.0% under-fill ratio and 10-s dynamic exclusion.

Liver. One microlitre of lipid extract was injected into a liver for liver samples (Thermo Scientific) by a Vanquish Split Sampler HT autosampler (Thermo Scientific). The LC system was coupled to a Q Exactive Focus mass spectrometer run by Tune software v.2.5.0.2042 (Thermo Scientific) by a HESI II heated ESI source kept at 300 °C (Thermo Scientific). The inlet capillary was kept at 300 °C, sheath gas was set to 25 units, auxiliary gas to 10 units, and the spray voltage was set to 5,000 V(+) and 4,000 V(−), respectively. The MS was operated in polarity switching mode acquiring positive and negative mode MS1 and MS2 spectra (Top2) during the same separation. MS acquisition parameters were 40,000 resolution, 1 x 10⁴ automatic gain control (AGC) target for MS1 and 1 x 10³ AGC target for MS2 spectra. The MS was operated in polarity switching dd-MS2 mode acquiring positive and negative mode MS1 and MS2 spectra (Top2 for positive, Top3 for negative mode) during the same separation. MS acquisition parameters were 60,000 resolution and 3 x 10⁴ AGC target for MS1 and 15,000 resolution and 5 x 10⁴ AGC target for MS2 scans, 100-μs MS1 and 35-μs MS2 ion accumulation time, 200- to 2,000-Th MS2 scan range, 1-Th isolation width for fragmentation, stepped HCD collision dissociation (HCD) collision energy (20, 30, 40 units), 1.0% under-fill ratio and 10-s dynamic exclusion.

Cells. Ten microlitres of lipid extract were injected through SII for Xcalibur by an Ultimate 3000 RSLC autosampler (Thermo Scientific). The LC system was coupled to a Q Exactive HF mass spectrometer run by Tune software v.2.8.0.2688 (Thermo Scientific) by a HESI II heated ESI source kept at 300 °C (Thermo Scientific). The inlet capillary was kept at 300 °C, sheath gas was set to 25 units, auxiliary gas to 10 units, and the spray voltage was set to 5,000 V(+) and 4,000 V(−), respectively. The MS was operated in polarity switching mode acquiring positive and negative mode MS1 and MS2 spectra (Top2 for positive, Top3 for negative mode) during the same separation. MS acquisition parameters were 60,000 resolution, 3 x 10⁴ AGC target for MS1 and 15,000 resolution and 5 x 10⁴ AGC target for MS2 scans, 100-μs MS1 and 200- to 1,600-Th MS1 scan range, 1-Th isolation width for fragmentation, fragmented HCD collision dissociation (HCD) collision energy (20, 30, 40 units), 1.0% under-fill ratio and 10-s dynamic exclusion.
peak equal to five were set. Last, for Group Unknown Compounds as well as Fill Gaps, mass tolerance was set to 10 ppm and retention time tolerance to 0.2 min. For best compound selection rules nos. 1 and 2 were set to unspecified, while MS1 was set to the selected MS order and MetaboScope was left in the preferred. For everything else, the default settings were used. Peak tables were exported as excel files in three levels of Compounds, Compound per File and Features (just Features for the ’Unaligned’) and later saved as csvs. In LipiDex Spectrum Searcher ’LipiDex_HCD_Acetate,’ LipiDex_HCD_Plants,’ LipiDex_Splash_ISTD_ Acetate,’ LipiDex_HCD_ULCFA’ and Ganglioside_20171205’ were selected as libraries for the DO while ’LipidBlot2_Reformatted_CoonLab,’ ‘LB cleaned’ and ’Lipid_Spectral_Library_20170523’ were selected for the FS. For the cells ’LipiDex_HCD_Acetate,’ LipiDex_HCD_Plants,’ LipiDex_HCD_ULCFA’ FAHA’ and ’Ganglioside_202000206’ were selected. Extended Data Fig 7 details the lipid classes searched for in these databases with their respective adducts. We further kept the defaults of 0.01 Th for MS1 and MS2 search tolerances, a maximum of one returned search result, and an MS2 low mass cutoff of 61 Th. Under the Peak Finder tab, Compound Discoverer was chosen as peak table type and its ’Aligned’ and ’Unaligned’ results, as well as the MS/MS results from Spectrum Researcher uploaded. Features had to be identified in a minimum of one file (four files for the FS), however, the average lipid identity was based on much higher average of 344 features found in plasma and 310 features in the liver dataset. We kept the defaults of a minimum of 75% of lipid spectral purity, an MS2 search dot product of at least 500 and reverse dot product of at least 700, as well as a multiplier of 2.0 (3.0 for FS) for the full width at half-maximum window, a maximum 15 ppm mass difference, adduct/dimer and in-source fragment filtering, and a maximum RT MAD Factor of 5.5. As post-processing all features that were only found in one file and had no identity were deleted and artefactual duplicates deleted.

For the FS liver dataset, peak areas were normalized to the 150:–18:1(7)-PC internal standard by dividing each peak area by the internal standards’ peak area of that sample and multiplying the result with the median of all internal standard peak areas. We also calculated the internal PC standard, for the entire DO dataset, in which many more LC–MS runs were collected, we used a batch correction approach (ComBat) to achieve normalization. In short, the ComBat method provided superior performance, especially in the case of the liver dataset. Specifically, the batch effects that occurred were easily and effectively corrected by application of the ComBat adjustment.

Before mapping analysis, the lipid metabolite data were adjusted for batch effects using the ComBat algorithm as implemented in the R’s’sva’ package. Batches correspond to sets of –32 samples each that were run on the same day on the mass spectrometer. Effectiveness of batch correction was confirmed by visualization of the first few principal components. We note that batch adjustment substantially increased the yield of QTL, even though no genotype information was included in the correction process. QTL mapping involves ‘scanning’ the genome and testing for association between the eight-state haplotype probabilities and the batch corrected MS feature levels. The genome scans were performed for each lipid metabolite feature using the scan1() function in R/qtl2 (ref. 15). This software fits a linear mixed model with sex and DO breeding generation as additive covariates and random effect to account for the kinship structure of the DO mice and computes a log10 likelihood ratio statistic (LOD) to evaluate the significance of the genetic effect at each pseudo-marker locus. Sex-specific genetic associations were identified using a separate set of genome scans that included a sex X genotype interaction in the linear mixed model. We identified suggestive QTL at LOD > 6.0 and notable QTL at LOD > 10.0. These threshold values were estimated by permutation analysis to obtain a family-wise error rate correction for genome-wide QTL search16. The family-wise error rate ensures that the maximum LOD score across the genome-wide search when applied to a trait with no QTL (that is, a permuted trait) will exceed the threshold with a fixed probability. For the lenient threshold 6.0, the genome-wide probability of false QTL detection is 0.20. For the stringent threshold 7.4, the genome-wide error rate is controlled at 0.05. The lenient threshold is used to identify the almost-notable associations that colocalize on the genome in hotspots.

Data analysis and plotting. Data analysis was largely performed using R v3.5.0 (ref. 17) in RStudio 6. Data formatting was performed using R/dplyr, 0.8.3 (ref. 18), R/ tidyverse_1.0.0 (ref. 19) and R/reshape2_1.3.4 (ref. 20) and visualizations were created using R/ggplot2_3.2.1 (ref. 21), R/ColorBrewer_1.1-2 (ref. 22), and for exploratory analysis, R/ggplotly_4.9.0 (ref. 20). Heatmaps were generated using R/ggmap_1.0.12 (ref. 20) and Manhattan plots were generated based on code accessible via the R gallery graph7. All boxplots were generated by ggplot2.geom_boxplot with the first and third quartiles (25th and 75th percentiles) for lower and upper hinges, 1.5x interquartile range for the length of the whiskers, centre line at median (50% quantile) and all raw data points, including outliers shown. Statistical tests were performed with MetaboAnalyst 4.0 (ref. 19). A 95% Bayesian confidence interval for each QTL was calculated using the function find_peaks() in R/qtl2.27 Human Mouse homologues were obtained from the MGI homology database (available here: http://www.informatics.jax.org/downloads/reports/HOM_MouseHomologSequence.rpt).

For each QTL generated using the scan1() function of R/ qtl2 (ref. 15). SNP associations were performed using the scan1np() function in R/qtl2_0.20 (ref. 20) accessing variants from the database cc_variants.sqlite (available here: https://ndownloader.figshare.com/files/18533432) and genes from mouse_ genes_mgi.sqlite (available here: https://ndownloader.figshare.com/files/17609253) via R/RSQTL_2.1.2 (ref. 7).

To conclude this genome-wide association analysis, we computed the Pearson’s correlation between the allele effect patterns for all cis-eQTL at a locus to which one or more lipids comapped. We performed the same calculation for hepatic cis-QTL identified in the previous study26. For example, at the chromosome 10 locus, we identified 25 QTL of unknown lipid identity in plasma and liver, all of which showed a strong NOD-driven allele effect pattern. About half of these QTL showed NOD as the high allele and half showed NOD as the low allele. We first computed the average allele effect pattern for the NOD high lipids and the NOD-low lipids. We then identified 55 cis-eQTL and 16 cis-pQTL that were within ±2 Mbp of the lipid QTL at ~127 Mbp on chromosome 10, and calculated the correlation between their allele effect patterns and the NOD high and the NOD-low lipid QTL. One gene showed a very strong correlation: B4galnt1. The overall correlation between the allele effects of the lipid QTL and the cis-eQTL or cis-pQTL was very low (for example, 0.0), suggesting that most expression traits are responding to genetic variants different from the lipid traits. However, the correlation between the lipid traits and either the expression or protein level for B4galnt1 was >0.97. As B4galnt1 is a known ganglioside, we then asked if the MS fragmentation pattern for the unknown lipids is consistent with gangliosides. It is worth noting that GM3 ganglioside standard (Cayman Chemicals, item no. 15587) contained N-acetyl-neuraminidate (NANA), the only sialic acid made by humans. All gangliosides observed in the DO samples contain N-glycolyl-neuraminidate (NGNA), a marine sialic acid in mice28. This powerful approach enabled us to combine the lipid data from one DO study with the gene expression and proteomic data of another DO study to nominate one candidate gene.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article. Data availability Genotypes and additional phenotype data associated with the DO mouse population have been deposited with Dryad (https://doi.org/10.5061/dryad.p105; data: Attie Islet eQTL data) (see Keller et al., ref. 26, for details). In addition, the data reported here are available for download and interactive web-based analysis at https://churchilllab.jax.org/qltviewer/islets. Genotyping used the Mouse Universal Genotyping Array (GigaMUGA; 143,259 markers). MS data have been deposited in Chorus (http://chorusproject.org/) under ID 1610 (direct links to cell experiments https://chorusproject.org/anonymous/download/experiment/498424520543749277.DOI live https://chorusproject.org/anonymous/download/experiment/a639bcb56024c1419a1d9f4340f9d26, DOI plasma https://chorusproject.org/anonymous/download/experiment/f0b723d2223462a492d0fdcdbe601b,Fs live https://chorusproject.org/anonymous/download/experiment/c930c419eb343d4b7d7f5308ebb96e, FS plasma https://chorusproject.org/anonymous/download/experiment/94dd025d100687924d40750d2e972cn). Human Mouse homologues were obtained from the Mouse Homology database (see Keller et al., ref. 32, for details). The data preparation and QTL mapping analysis are reproducibly documented in UNIX shell and R scripts posted on github (https://github.com/dmgatti/NatureResearch_2020_scripts and https://github.com/vanilink/DOLipids with input from Supplementary Tables 8 and 9. The genome –lipid associations are also accessible through an interactive web-based
analysis tool that will allow users to replicate the analyses reported here (http://lipidgenie.com/). The source code for this resource can be found at https://github.com/coongroup/LipidGenie.

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Author contributions

J.D.R., M.P.K., G.A.C., A.D.A. and J.J.C. designed the experiment. K.L.S., D.S.S., M.E.R. and M.P.K. assisted with sample collection. V.L., P.D.H., E.A.T. and T.R.R. performed the MS analysis. E.M.C. performed cell experiments. V.L., I.J.M., D.R.B., P.D.H., M.P.K., D.M.G., G.R.K., D.P. and G.A.C. analysed data. V.L., K.A.O., I.J.M., M.P.K., K.W.B., G.A.C., A.D.A. and J.J.C. wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to J.J.C.

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Extended Data Fig. 1 | Identified lipids and unidentified features occupy characteristic regions in the m/z vs. RT space. a, In plasma, we quantified 1,721 lipidomic features, 621 of which were identified, and b, In liver, we quantified 1,562 lipidomic features, 615 of which were identified. Abbreviations: m/z (mass-to-charge), RT (retention time).
Extended Data Fig. 2 | Lipid profiling and subsequent QTL mapping reveals clusters of associated lipids. **a**, Lipid class distribution of all 1,721 plasma and **b**, 1,562 liver lipidomic features. **c**, 1,405 plasma and **d**, 1,190 lipid features showed at least one QTL with an LOD > 6 as displayed in a Manhattan plot (n = 3,353 and 2,269 total QTL, respectively). Hierarchical clustering of these features against the 69,005 markers on the mouse genome, resulted in clustering of lipid class based on hotspots at the genetic level. Abbreviations: Chr (chromosome), DO (diversity outbred), QTL (quantitative trait loci), LOD (logarithm of odds).
Extended Data Fig. 3 | Apoa2 as the candidate gene at the largest lipid hotspot. a, 255 plasma (black) features mapping to the apoa2 locus on chromosome 1 share an allele effect pattern with upregulation in the 129 allele, while 2 mapping liver features (white) do not share the pattern (based on hierarchical clustering on allele effects, with a Euclidean distance cutoff of h = 1.5). b, The allele effect is exemplary replicated in an independent experiment of founder strain plasma CE(18:2) levels (n = 4 for each sex and strain, boxplots are defined with the first and third quartiles (25th and 75th percentile) for lower and upper hinges, 1.5x interquartile range for the length of the whiskers, centre line at median (50% quantile)). c, The same pattern was not visible in previously reported Apoa2 liver protein and RNA allele effects. Abbreviations: CE (cholesteryl ester), FS (founder strain).
Extended Data Fig. 4 | B4galnt1 as the candidate gene at the hotspot with the largest LOD. a, The selection of B4galnt1 as the candidate gene for the chromosome 10:127 Mbp locus was corroborated by NOD-specific allele effects in previously reported liver eQTL and b, pQTL. c, The allele effect patterns of the later as gangliosides identified features mapping to the B4galnt1 locus could further be validated in an independent experiment of founder strain mice (exemplar GM3 pattern, n = 4 for each sex and strain, boxplots are defined with the first and third quartiles (25th and 75th percentile) for lower and upper hinges, 1.5x interquartile range for the length of the whiskers, center line at median (50% quantile)). Abbreviations: FS (founder strain), Mbp (megabase pair).
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Allele effects characterize genome-lipid hotspots. 

**a**, Hierarchical clustering of allele effects at Chr 6:91 Mbp resulted in 21 features with matching A/J down effect (main cluster featuring the six B6 male specific features (red) after row-scaling and Ward clustering, cutoff at h=5). 

**b**, Consistently, the pattern of male >> female was observed for each of the FS except for A/J as visible in the example for m/z 1130 (n = 4 for each sex and strain, boxplots are defined with the first and third quartiles (25th and 75th percentile) for lower and upper hinges, 1.5x interquartile range for the length of the whiskers, center line at median (50% quantile).)

**c**, Hierarchical clustering of allele effects at Chr 5:31 Mbp locus resulted in 10 features with matching B6 and NZO up effect (main cluster featuring LysoPC 14:0 (turquoise) after row-scaling and Ward clustering, cutoff at h=8). 

**d**, This pattern could be replicated in the FS (n = 4 for each sex and strain, boxplots are defined with the first and third quartiles (25th and 75th percentile) for lower and upper hinges, 1.5x interquartile range for the length of the whiskers, center line at median (50% quantile)), as shown for LysoPC 14:0, as well as 

**e**, in opposite directionality in a liver eQTL. 

**f**, Hierarchical clustering of allele effects at Chr 7:79 Mbp locus resulted in 8 features with matching WSB down effect (main cluster featuring PUFA-containing phospholipids (turquoise) after row-scaling and Ward clustering, cutoff at h=2.5). 

**g**, The mapping phospholipids contained polyunsaturated fatty acids such as 20:4 and 22:6. 

**h-i**, Abhd2 liver RNA and protein allele effects matched with an opposite WSB high effect. Abbreviations: DO (diversity outbred), FS (founder strain), Chr (chromosome), Mbp (megabase pair), PC (phosphatidylcholine), PUFA (polyunsaturated fatty acid).
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Overexpressing ABHD1 and ABHD3 results in distinct phospholipid signature. a, Experimental design of the validation experiment featuring three technical and four biological replicates of Hepa1-6 cells either untransfected (CTL), transfected with a His-tag GFP control (GFP), or transfected with MYC-tagged ABHD1 or ABHD3. b, Western blot of Hepa1-6 overexpression of ABHD1 and ABHD3. Shown is an overlay of membrane and ECL blot for MYC-tag. c, Heatmap of top 49 features from discovery lipidomics experiment with p < 0.05 (ANOVA, Fisher’s LSD post-hoc). Features were sum-normalized and log2-transformed. Hierarchical clustering (Ward clustering, Euclidean distance) shows two clusters with opposite fold changes distinguishing between ABHD1 and ABHD3 and the GFP control.
| Abbreviation | Lipid Class | Adduct(s) |
|--------------|-------------|-----------|
| AC           | Acyl Carnitine | [M+H]⁺ |
| Alkanyl-TG   | Alkanyl Triacylglycerol | [M+NH₄]⁺ |
| Alkenyl-TG   | Alkenyl Triacylglycerol | [M+NH₄]⁺ |
| Alkenyl-DG   | Alkenyl Diacylglycerol | [M+H]⁺ |
| CE           | Cholesteryl ester | [M+NH₄]⁺ |
| Cer [AP]     | CeramideAP | [M-H]⁻; [M+Ac-H]- |
| Cer [AS]     | CeramideAS | [M+Ac-H]-; [M-H]⁻ |
| Cer [BS]     | CeramideBS | [M-H]⁻; [M+Ac-H]- |
| Cer [NP]     | CeramideNP | [M-H]⁻; [M+Ac-H]- |
| Cer [NS]     | CeramideNS | [M+H]⁺; [M+Ac-H]-; [M-H]⁻; [M+H-H₂O]⁺ |
| CerP         | Ceramide-1-Phosphate | [M+H]⁺; [M-H]⁻ |
| CL           | Cardiolipin | [M-H]⁻; [M-2H]²⁻ |
| DG           | Diacylglycerol | [M+NH₄]⁺ |
| DGDG         | Dihexosyldiacylglycerol | [M+H]⁺; [M-H]⁻; [M+Ac-H]- |
| FA           | Fatty acid | [M-H]⁻ |
| GD2-NGNA     | GD2-Ganglioside-N-Glycolytinearminic acid | [M+H]⁺ |
| GD3-NGNA     | GD3-Ganglioside-N-Glycolytinearminic acid | [M+H]⁺ |
| GM1-NGNA     | GM1-Ganglioside-N-Glycolytinearminic acid | [M+H]⁺ |
| GM2-NANA     | GM2-Ganglioside-N-Acetyleneuraminic acid | [M+H]⁺ |
| GM2-NGNA     | GM2-Ganglioside-N-Glycolytinearminic acid | [M+H]⁺ |
| GM3-NANA     | GM3-Ganglioside-N-Acetyleneuraminic acid | [M+H]⁺ |
| GM3-NGNA     | GM3-Ganglioside-N-Glycolytinearminic acid | [M+H]⁺ |
| HexCer [AP]  | Hexosyl CeramideAP | [M+Ac-H]- |
| HexCer [NS]  | Hexosyl CeramideNS | [M+H]⁺; [M-H]⁻; [M+Ac-H]- |
| LysOPE       | Lysophosphatidylethanolamine | [M+H]⁺; [M-H]⁻ |
| LysPG        | Lysophosphatidylglycerol | [M-H]⁻ |
| LysP1        | Lysophosphatidylinositol | [M-H]⁻ |
| LysPS        | Lysophosphatidyl serine | [M-H]⁻ |
| Lysosmycin   | Lysosphingomyelin | [M+H]⁺; [M+Ac-H]- |
| Methyl-PA     | Methylphosphatic Acid | [M-H]⁻ |
| MGDG         | Monohexosyldiacylglycerol | [M+NH₄]⁺; [M+Ac-H]- |
| PA           | Phosphatic acid | [M+NH₄]⁺; [M-H]⁻ |
| PC           | Phosphatidyocholine | [M+H]⁺; [M+Ac-H]- |
| PE           | Phosphatidylethanolamine | [M+H]⁺; [M-H]⁻ |
| PE-NMe       | Monomethyl Phosphatidylethanolamine | [M+H]⁺; [M-H]⁻ |
| PE-NMe2      | Dimethyl Phosphatidylethanolamine | [M+H]⁺; [M-H]⁻ |
| PG           | Phosphatidylglycerol | [M+NH₄]⁺; [M-H]⁻ |
| PI           | Phosphatidylinositol | [M-H]⁻; [M+NH₄]⁺; [M+H]⁺ |
| Plasmanyl-PC | Plasmanyl Phosphatidycholine | [M+H]⁺; [M+Ac-H]- |
| Plasmanyl-PE | Plasmanyl Phosphatidylethanolamine | [M-H]⁻ |
| Plasmenyl-PC | Plasmenylphosphatidylcholine | [M+H]⁺; [M+Ac-H]- |
| Plasmenyl-PE | Plasmenylphosphatidylethanolamine | [M+H]⁺; [M-H]⁻ |
| PS           | Phosphatidylserine | [M+H]⁺; [M-H]⁻ |
| S1P          | Sphingosine-1-Phosphate | [M+H]⁺; [M-H]⁻ |
| SHexCer      | Sulfatides | [M+H]⁺; [M-H]⁻ |
| SM           | Sphingomyelin | [M+H]⁺; [M+Ac-H]- |
| SP           | Sphingosine | [M+H]⁺; [M+H-H₂O]⁺ |
| SQDG         | Sulfoglucosylsphingomyelin | [M+NH₄]⁺; [M-H]⁻ |
| TG           | Triacylglycerol | [M+NH₄]⁺; [M+Na]⁺ |

Extended Data Fig. 7 | Lipid class abbreviations and identifications with respective adduct types. As searched for in LipiDex databases (see Methods).
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- A description of all covariates tested
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- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Software and code

Policy information about availability of computer code

Data collection

Plasma. Ten microliters of lipid extract were injected through SII for Xcalibur by an Ultimate 3000 RSLC autosampler (Thermo Scientific) coupled to a Q Exactive Focus mass spectrometer run by Tune software version 2.5.0.2042 (Thermo Scientific).

Liver. One microliter of lipid extract was injected through SII for Xcalibur (Thermo Scientific) by a Vanquish Split Sampler HPLC autosampler (Thermo Scientific) coupled to a Q Exactive HF mass spectrometer run by Tune software version 2.8.0.2688 (Thermo Scientific).

Cells. Ten microliters of lipid extract were injected through SII for Xcalibur (Thermo Scientific) by a Vanquish Split Sampler HPLC autosampler (Thermo Scientific) coupled to a Q Exactive HF mass spectrometer run by Tune software version 2.9.3.2948 (Thermo Scientific).

Data analysis

The resulting LC-MS lipidomics raw files were converted to mgf files via MSCIConvertGUI (ProteoWizard, Dr. Parag Mallick, Stanford University) and processed using Compound Discoverer 2.0 (Thermo Fisher Scientific) and an in-house developed open-source software suite, LipDex. The quantification of the internal standard was obtained through TraceFinder 4.0 (Thermo Fisher Scientific).

Data analysis was largely performed using R in RStudio. The lipid metabolite data were adjusted for batch effects using the Combat algorithm as implemented in the Rsva software package. The genome scans were performed using the scan1() function in R/qtl2. Data formatting was performed utilizing R/dplyr_0.8.3, R/rdf_1.0.0 and R/reshape2_1.4.3 and visualizations were created using R/ggplot2_3.2.1, R/RCColorBrewer_1.1.2, and for exploratory analysis, R/plotly_4.9.0. Heatmaps were generated using R/ggheatmap_1.0.12 and Manhattan plots were generated based on code accessible via the R graph gallery. All boxplots were generated by ggplot2:geom_boxplot. A 95% Bayesian confidence interval (CI) for each QTL was calculated using the function find_peaks() in R/qtl2. Allele effects for each QTL were generated using the scan1blup() function of R/qtl2. SNP associations were performed using the scan1snp() function in R/qtl2_0.20 accessing variants from the database cc_variants.sqlite (available here: https://ndownload.itigshare.com/files/18533342) and genes from mouse_gens_mgi.sqlite (available here: https://ndownload.itigshare.com/files/17609252) via R/RSQLite_2.1.2.

The data preparation and R calculation and mapping analysis are reproducibly documented in UNIX shell and R scripts posted on github (https://github.com/dmgartt/AttleMetabolomics). Code for data analysis and plotting is available at https://github.com/vanlink/DOLipids/ with input from Supplementary Tables S8 and S9. The genome-lipid associations are also accessible through an interactive web-based analysis tool that will allow users to replicate the analyses reported here (http://lipidgenie.com/). The source code for this resource can be found at https://
Data

Policy information about availability of data. All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
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Genotypes and additional phenotype data associated with the DO mouse population have been deposited with Dryad (doi:10.5061/dryad.pj05; data files: Attie Islet eQTL data). In addition, the data reported here are available for download and interactive web-based analysis at https://churchilllab-jax.org/qtviewer/attie/islets. Genotyping used the Mouse Universal Genotyping Array (GigaMUGA; 143,259 markers).

Mass spectrometry data have been deposited in Chorus [http://chorusproject.org/] under ID 1610 [direct links to cell experiments https://chorusproject.org/anonymous/download/experiment/498424520543479277, DO liver https://chorusproject.org/anonymous/download/experiment/a6392cc5602c441c9a1df94f4340db2b, DO plasma https://chorusproject.org/anonymous/download/experiment/f8b273d22236f2a3d92cfdd0eb601b6, FS liver https://chorusproject.org/anonymous/download/experiment/c930d419eb34defebda7f53508c6969e, and FS plasma https://chorusproject.org/anonymous/download/experiment/9a4a275df011468792440767fca97ca]. Human Mouse homologues were obtained from the MGI homology database [available here: http://www.informatics.jax.org/downloads/reports/HOM_MouseHumanSequence.rpt] and genes from mouse_genes_mgi.sqlite [available here: https://ndowloder.figshare.com/files/18533342] and genes from mouse_genes_mgi.sqlite [available here: https://ndowloder.figshare.com/files/17609252].

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

A sample size of four waves of 100 mice was chosen. A total of n=384 mice were sacrificed at ~22 weeks of age. This cohort of mice analyzed for this study existed and was previously described.[32, 33, 72]

32. Keller, M. P. et al. Genetic Drivers of Pancreatic Islet Function. Genetics 209, 335–356 (2018).
33. Keller, M. P. et al. Gene loci associated with insulin secretion in islets from nondiabetic mice. Journal of Clinical Investigation vol. 129 4419–4432 (2019).
37. Mitock, K. A. et al. Islet proteomics reveals genetic variation in dopamine production resulting in altered insulin secretion. Journal of Biological Chemistry vol. 293 5860–5877 (2018).

For the cell validation experiments, a sample size of n=12 was chosen (n=3 biological replicates x n=4 technical replicates) for each mutant and control based on previous experience with the minimum sample size required for MS validation experiments. Sample size calculation was not performed, but deemed sufficient for the purpose upon observing statistical significance in data analysis.

Data exclusions

No data were excluded from the analyses.

Replication

“Although there is no biological replication of genetically identical animals in an outcross population, there is replication of genotypes at specific loci. This local genetic replication enables one to link phenotype with genotype, as in a human GWAS or QT mapping studies in DO mice.”[32]

32. Keller, M. P. et al. Genetic Drivers of Pancreatic Islet Function. Genetics 209, 335–356 (2018).

In addition, the founder strain (FS) data set represents a way of confirming or supporting the gene-lipid associations found in the DO. Several of such instances are highlighted in the manuscript, while the full FS data set is provided in the supplement to enable others to use the founder strain data for additional examples not discussed in the manuscript.

As noted above, cell validation experiments were performed in biological and technical replicate.

For all MS experiments, subsets of samples were re-analyzed as technical replicates on the LC-MS platform and the results successfully reproduced (i.e. analysis reproducibility).

Randomization

Mice were allocated by waves of 100 each with an equal number of males and females. Samples were randomized into batches for sample preparation and randomized again for running on the LC-MS system. Cell samples were also randomized for sample preparation and randomized again for running on the LC-MS system.

Blinding

Blinding was not relevant to this study as there were no groups.
Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☐   | Antibodies            |
| ☒   | Eukaryotic cell lines |
| ☐   | Palaeontology and archaeology |
| ☒   | Animals and other organisms |
| ☒   | Human research participants |
| ☐   | Clinical date         |
| ☐   | Dual use research of concern |

### Antibodies

**Antibodies used**
- Rabbit anti-MYC antibody, CST, 2278 clone 71D10, lot: 5
- Goat anti-rabbit-HRP conjugated antibody, CST, 7074S, lot: 24

**Validation**
The company validated the antibody and expects reactivity against all species since the tag is a humanized MYC that would not otherwise be present in our Hela-1 cells. In-house, we further validated by running untransfected control cells, and cells transfected with an untagged control plasmid (GFP) as comparison.

*"Myc-tag (71D10) Rabbit mAb detects recombinant proteins containing the Myc epitope tag. The antibody recognizes the Myc-tag fused to either the amino acid or carboxy terminus of targeted proteins in transfected cells. During the production process, side by side comparisons are performed between the new antibody lot and the previous lot. New lots are not released for sale unless the performance is equal to or better than the previous lot in each application. In our tests of the #2278 antibody, we were able to detect Myc-Akt in our transfected cell lines. No signal was observed in the non-transfected controls."

The Myc epitope tag is widely used to detect expression of recombinant proteins in bacteria, yeast, insect and mammalian cell systems. [Munro, S. and Pelham, H.R. (1984) EMBO J 3, 3087-93]*

See manufacturer’s note here: https://www.cellsignal.com/products/primary-antibodies/myc-tag-71d10-rabbit-mab/2278

### Eukaryotic cell lines

**Policy information about cell lines**
- Hepa1-6 cells [ATCC® CRL-1830]

**Authentication**
- We did not authenticate in-house. Cells were purchased directly from ATCC by J. Simcox, and propagated for use in our lab.

**Mycoplasma contamination**
- We did save scraped cell RNA to test for mycoplasma but could not run the qPCR due to the lab shutdown.

**Commonly misidentified lines**
- No commonly misidentified cell lines were used in this study.

### Animals and other organisms

**Policy information about studies involving animals**: ARRIVE guidelines recommended for reporting animal research

**Laboratory animals**

Equal numbers of male and female Diversity Outbred [DO] mice and the eight founder strains [C57BL/6J [86], A/J, 129S1/SvImJ (129), NOD/ShiLtJ (NOD), NZO/HILJ (NZO), PWK/PhJ (PWK), WSB/Ei [WSB], and CAST/Ei [CAST]] were all obtained from the Jackson Labs and have been previously described [32,33,72]. Briefly, all mice were housed within the vivarium at the Biochemistry Department, University of Wisconsin-Madison, and maintained on a Western-style high-fat/high-sucrose (HF/HS) diet (44.6% kcal fat, 34% carbohydrate and 17.3% protein) from Envigo Teklad (TD.08811) for 36 weeks. All mice were maintained in a temperature and humidity-controlled room on a 12 hr light/dark cycle (lights on at 6AM and off at 6PM), and provided water ad libitum. At ~22 weeks of age, mice were sacrificed following a 4 hr fast.

32. Keller, M. P. et al. Genetic Drivers of Pancreatic Islet Function. Genetics 209, 355–356 (2018).
33. Keller, M. P. et al. Gene loci associated with insulin secretion in islets from nondiabetic mice. Journal of Clinical Investigation vol. 129 4419-4432 (2019).
72. Mtock, K. A. et al. Islet proteomics reveals genetic variation in dopamine production resulting in altered insulin secretion. Journal of Biological Chemistry vol. 293 5860-5877 (2018).

**Wild animals**
The study did not involve wild animals.
| Field-collected samples | The study did not involve samples collected from the field. |
|-------------------------|-------------------------------------------------------------|
| Ethics oversight        | All experiments involving mice were preapproved by an AAALAC-accredited institutional Animal Care and Use Committee of the College of Agriculture and Life Sciences (CALS) at the University of Wisconsin-Madison. The CALS Animal Care and Use Protocol number associated with the study is A005822, A. D. Attie, Principal Investigator. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.