The genomic architecture of blood metabolites based on a decade of genome-wide analyses

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Abstract

Metabolomics examines the small molecules involved in cellular metabolism. Approximately 50% of total phenotypic differences in metabolite levels is due to genetic variance, but heritability estimates differ across metabolite classes and lipid species. We performed a review of all genetic association studies, and identified > 800 class-specific metabolite loci that influence metabolite levels. In a twin-family cohort (N = 5,117), these metabolite loci were leveraged to simultaneously estimate total heritability (h^2_{total}), and the proportion of heritability captured by known metabolite loci ($h^2_{Metabolite\text{-}hits}$) for 309 lipids and 52 organic acids. Our study revealed significant differences in $h^2_{Metabolite\text{-}hits}$ among different classes of lipids and organic acids. Furthermore, phosphatidylcholines with a high degree of unsaturation had higher $h^2_{Metabolite\text{-}hits}$ estimates than phosphatidylcholines with a low degree of unsaturation. This study highlights the importance of common genetic variants for metabolite levels, and elucidates the genetic architecture of metabolite classes and lipid species.

50 The metabolome is defined as the collection of metabolites, i.e., small molecules involved in cellular
51 metabolism, which are produced in cells[1] and consist of many classes[2–5]. The overall aim of
52 metabolomics is to provide a holistic overview of the metabolome[1], and its role in biological
53 mechanisms and metabolic disturbances in diseases. Elucidating this role may offer new therapeutic
54 targets or new biomarkers for disease diagnosis[6]. Variation in metabolite levels can arise due to gender[7],
55 and age[8], as well as physiologic effects, behavior, and lifestyle factors, such as diet[9]. Genetic differences
56 may be a source of direct variation in metabolomics profiles, or an indirect source of variation through
57 genetic influences on physiology, behavior, and (or) lifestyle.

58 Genome- and metabolome-wide analysis of common genetic variants in human metabolism
59 have successfully identified genetically influenced metabolites[10]. In 2008, the first genome-wide
60 association study (GWAS; N = 284 participants) identified four genetic variants associated with
61 metabolite levels[11]. Thereafter, GWAS with increasing sample sizes, and in diverse populations,
62 identified hundreds of Single Nucleotide Polymorphism (SNP) associations with metabolites from a wide
63 range of metabolite classes[10]. Additional metabolite loci have been identified by leveraging
64 low-frequency and rare-variant analyses using (exome-) sequencing. We conducted a comprehensive
65 review of all quantitative trait locus (QTL) discovery for metabolites and supply the complete reference
66 list in **Supplementary Note 1**.

67 Twin and family studies have established that the heritability (h^2; proportion of phenotypic
68 variance due to genetic factors) of metabolite levels is 50% on average, with a range from h^2 = 0% to h^2 =
69 80%[9,12–19]. Several studies reported differences in heritability estimates among different classes of lipid
70 species[16,18] or lipoprotein subclasses[17]. For example, Rhee et al. (2013) reported higher heritability
71 estimates for amino acids than for lipids[15]. Essential amino acids, which cannot be synthesized by an
72 organism *de novo*[20], had lower heritability than non-essential amino acids[15], that are synthesized within

73 the body[20]. Several techniques are available to estimate the contribution of measured SNPs to trait
74 heritability[21], and, given SNP data in family members, to simultaneously estimate SNP-associated (h^2_{SNP})
75 and pedigree-associated genetic variance (h^2_{ped})[22]. Together the SNP- and pedigree-associated genetic
76 effects account for the narrow-sense heritability. However, when including data of family members, the
77 variance explained by genetic effects (h^2_{total}) may be biased upwards by shared environmental factors
78 and/or non-additive genetic effects [22,23].

79 An improved understanding of the genetic architecture of metabolites will benefit our
80 understanding of the aetiology of diseases and traits, such as cardiometabolic diseases[24], migraine[25],
81 psychiatric disorders[26], and cognition[27]. Here we aim to further our understanding of the contribution of
82 genetic factors to variation in fasting blood metabolic measures (henceforth referred to as *metabolites*
83 for brevity) by the analysis of data from multiple metabolomics platforms in a large cohort of twins and
84 family members (N = 5,117). Specifically, we aim to estimate the total genetic variance of metabolite
85 levels (h^2_{total}), and to elucidate the contribution to metabolite levels of known metabolite class-specific
86 and metabolite class-unspecific loci ($h^2_{Metabolite\text{-}hits}$), on the basis of the results of a decade of GWA and
87 (exome-) sequencing studies (**Supplementary Data 1**). To this end, we characterized all published
88 metabolite-SNP associations by metabolite classification, and used linear-mixed models to estimate the
89 h^2_{total}, h^2_{SNP} and $h^2_{Metabolite\text{-}hits}$ simultaneously for 369 metabolites (**Figure 1**). In these models, the
90 $h^2_{Metabolite\text{-}hits}$ consists of two variance components, a component attributable to metabolite loci
91 associated with metabolites of a specific superclass ($h^2_{Class\text{-}hits}$) and a component attributable other
92 metabolite loci ($h^2_{Notclass\text{-}hits}$; **Figure 1**). We further expand on the current knowledge of the genetic
93 aetiology of metabolite classes by employing mixed-effect meta-regression models to test differences in
94 heritability estimates among metabolite classes and among lipid species.

95 Intriguingly, phosphatidylcholines[14] and triglycerides (TGs)[19] show increasing heritability with
96 increasing number of carbon atoms and/or double bonds in their fatty acyl side chains. Draisma et. al
97 speculated this might be attributable to differences in the number of metabolic conversion rounds for
98 phosphatidylcholines or TGs with a variable number of carbon atoms[14]. To distinguish between the
99 effects of the number of carbon atoms or number of double bonds in the fatty acyl side chains of
100 phosphatidylcholines and TGs, we conducted additional univariate follow-up analyses.

101 # Results

102 ## Metabolite classification

103 In the period of November 2008 to October 2018, 40 GWA and (exome-) sequencing studies identified
104 242,580 metabolite-SNP or metabolite ratio-SNP associations (see **Supplementary Note 1**). All 242,580
105 associations may be found at: http://bbmri.researchlumc.nl/atlas/#data,which lists the significant SNP-
106 metabolite associations by study. These associations included 1,804 unique metabolites or ratios, and
107 49,231 unique SNPs (43,830 after converting all SNPs to NCBI build 37; **Supplementary Data 1**). The
108 Human Metabolome Database (HMDB)[2–5] identifiers of each metabolite were retrieved in order to
109 extract information concerning the metabolite's hydrophobicity and chemical classification (see
110 **Methods**). Excluding the ratios and unidentified metabolites, we classified 953 metabolites into 12
111 'super classes' (**Table 1**), 43 'classes', or 77 'subclasses' based on the HMDB classification
112 (**Supplementary Data 1**). The majority of the metabolites were classified into the super classes lipids or
113 organic acids. The lipids could be subdivided into 8 classes, with 1 to 95,795 metabolite-SNP associations
114 per class (mean = 17,589; SD = 32,553), and in 32 subclasses, with the number of subclass metabolites-
115 SNP associations ranging from 1 to 40,440 (mean = 4,673; SD = 9,124). The organic acids and derivatives
116 were divided in 9 classes, with the number of metabolite-SNP associations ranging from 1 to 26,832

117 (mean = 3,374; SD = 8,832). The organic acids and derivatives were also divided into 17 organic acid
118 subclasses, with the number of subclass metabolite-SNP associations ranging from 1 to 26,448 (mean =
119 1,786; SD = 6,371; **Supplementary Data 1**). Across all four platforms 427 metabolites were assessed.
120 After excluding the ratios (17) and the metabolites of super classes not included in the curated
121 metabolite-SNP association list (8), data were available for 402 metabolites. The full list of metabolites,
122 with their classifications and the quartile values of the untransformed levels, are included in
123 **Supplementary Table 1**. The 402 metabolites were classified as 336 lipids, 53 organic acids, 9 organic
124 oxygen compounds, 3 proteins and one organic nitrogen compound, these super classes were consisted
125 of 12 classes (**Supplementary Table 2**). In this paper we mainly focus on the first two super classes. After
126 quality control (QC), 369 metabolites from these two super classes were retained for analysis.

127 Characterization of the heritable influences on lipid and organic acid levels

128 Data of 5,117 participants were available from the following four metabolomics platforms: the
129 Nightingale Health ^{1}H-NMR platform, a UPLC-MS Lipidomics platform, the Leiden ^{1}H-NMR platform, and
130 the Biocrates Absolute-IDQ™ p150 platform. The participants were registered with the Netherlands
131 Twin Register (NTR)[28] and were clustered in 2,445 nuclear families. Metabolomics and SNP data were
132 available for all participants. Background and demographic characteristics for the sample can be found
133 in **Table 2**.

134 We aimed to assess the variance explained by previously identified metabolite GWA and
135 (exome-) sequencing genetic variants in our (independent) sample. Clearly, our results are conditional
136 on the power of past the studies, as the list of metabolite genetic variants is based on previous GWA and
137 (exome-) sequencing studies, which vary in power. We present the sample size of each past study in
138 **Supplementary Note 1**, and the sample size per metabolite-SNP association in **Supplementary data 1**.

139 Linear-mixed models including all loci for genetic variants associated with metabolites in a single
140 genetic relatedness matrix (GRM) will contain SNPs that are associated with some metabolites, but not
141 with others, or include many SNPs that are not associated with a given metabolite. We therefore
142 created two GRMs for the loci associated with metabolite hits (see **Methods**): one class-specific and one
143 non-class specific (i.e. GRMs including metabolite loci for all metabolites, except for the target
144 metabolite class). We explored models for the 12 class-specific and the corresponding not-class specific
145 GRMs (**Supplementary Note 2**). These models displayed high degrees of non-convergence (37.9% total),
146 with models including small class-specific GRMs displaying more non-convergence (**Supplementary**
147 **Table 2**). Therefore, the results in the remainder of this paper were based on the metabolite super
148 classes, i.e. lipids and organic acids.

149 For the 369 lipids and organic acids, we carried out unconstrained four-variance component
150 analyses (**Figure 1**). In genome-wide complex trait analysis (GCTA)[21] we specified a model in which we
151 partition the metabolite variation into SNP-associated (h^2_{SNP}), pedigree-associated (h^2_{ped}), class-specific
152 metabolite-loci-associated ($h^2_{class\text{-}hits}$), and not-class metabolite-loci-associated ($h^2_{notclass\text{-}hits}$) genetic
153 variation (**Figure 1**). We report the total heritability (h^2_{total}), the proportion attributable to metabolite
154 superclass-specific loci ($h^2_{Class\text{-}hits}$), the proportion of variance attributable to non-superclass metabolite
155 loci ($h^2_{Notclass\text{-}hits}$) and the contribution of known metabolite loci to metabolite levels ($h^2_{Metabolite\text{-}hits}$). The
156 analyses were performed separately for lipids and organic acids, with class-specific and corresponding
157 non-class GRMs (created using the LDAK program[29,30]) in both sets of analyses. The lipid analyses
158 employed a class-specific GRM of 479 lipid loci and a corresponding non-class GRM of 596 loci
159 (**Supplementary Figure 1**). The organic acid analyses included a class-specific GRM of 397 loci and a non-
160 class GRM of 683 loci (**Supplementary Figure 1**). Before the analyses, the metabolite data were
161 normalized (log-normal or inverse rank; **see Methods**). All models included age at blood draw, sex, the

162 first 10 principal components (PCs) from SNP genotype data, genotyping chip and metabolomics
163 measurement batch as covariates.

164 **Supplementary Table 3** includes the estimates from the four-variance genetic component
165 models for all 369 metabolites. The genomic relatedness matrix residual maximum likelihood (GREML)
166 algorithm converged for 361 (97.8%) of the 53 organic acids and 316 lipids (**Supplementary Table 4**).
167 Non-convergence of the GREML algorithm was observed for 6 metabolites (1.6%). The analyses of 2
168 metabolites (0.5%) were not completed due to non-invertible variance-covariance matrices. The
169 estimates for h^2_{total} of the 309 lipids ranged from 0.11 to 0.66 (mean = 0.47; mean s.e. = 0.04). The
170 estimates for $h^2_{Metabolite\text{-}hits}$ ranged from -0.05 to 0.16 (mean = 0.06; mean s.e. = 0.03; **Table 3**). The 52
171 organic acids had h^2_{total} estimates ranging from 0.14 to 0.72 (mean = 0.41; mean s.e. = 0.04). The
172 estimates for $h^2_{Metabolite\text{-}hits}$ ranged from -0.08 to 0.11 (mean = 0.01; mean s.e. = 0.02; **Table 3**). On
173 average, for both lipids and organic acids the h^2_{class} was higher than the $h^2_{Notclass}$, with $h^2_{Class\text{-}hits}$ ranging
174 from -0.02 to 0.16 (0.06; mean s.e. = 0.02) for lipids and from -0.04 to 0.14 for organic acids (mean =
175 0.01; mean s.e. = 0.02). For both lipids and organic acids $h^2_{Notclass\text{-}hits}$ was zero (mean s.e. = 0.02), ranging
176 from -0.06 to 0.12 for lipids and from -0.06 to 0.05 for organic acids (**Table 3**).

177 Including multiple metabolomics platforms allowed for a comparison of metabolites as
178 measured on multiple platforms. An earlier study showed that 29 out of 43 metabolites present on two
179 platforms to exhibit moderate heritability on both platforms[31]. In the current study, 61 metabolites were
180 measured on multiple platforms (phenotypic correlations provided in **Supplementary Table 5**), with
181 moderate h^2_{total} on each of the platforms and on average a positive correlation of 0.36 between the
182 h^2_{total} of the same metabolite assessed on different platforms (**Supplementary Table 5**).

183 ## Differential heritability among metabolite classes and lipid-species

184 **Figure 2** shows variation in median heritability among the following classes of organic acids: keto acids,
185 hydroxy acids and carboxylic acids (see **Supplementary Table 1** for metabolites per class). Keto acids,
186 followed by carboxylic acids, had the highest median h^2_{total}, and $h^2_{Class\text{-}hits}$ estimates (**Figure 2**). While
187 hydroxy acids had the highest median $h^2_{Notclass\text{-}hits}$ and $h^2_{Metabolite\text{-}hits}$ estimates, the lowest median h^2_{total},
188 and $h^2_{Class\text{-}hits}$ estimates were observed for these metabolites (**Figure 2**). To investigate whether
189 heritability differs significantly among classes of organic acids, we applied multivariate mixed-effect
190 meta-regression, corrected for metabolite platform effects (see **Methods**). The multivariate mixed-
191 effect meta-regression models showed that h^2_{total} and $h^2_{Class\text{-}hits}$ for the organic acid classes did not differ
192 significantly. However , significant differences among the organic acid classes, though, were observed
193 with respect to the $h^2_{Metabolite\text{-}hits}$ estimates ($F(4, 47) = 3.44$, FDR-adjusted p-value = 0.03), and the $h^2_{Notclass\text{-}}$
194 $_{hits}$ estimates ($F(4,47) = 19.95$, FDR-adjusted p-value = $1.25\text{x}10^{-08}$; **Supplementary Table 6**).

195 The multivariate mixed-effect meta-regressions were also applied to assess the significance of
196 heritability differences among essential and non-essential amino acids (subdivision of carboxylic acids;
197 see **Supplementary Table 7**) and among lipid classes (see **Supplementary Table 1** for metabolites per
198 lipid class). The meta-regression analyses revealed no significant mean differences among essential and
199 non-essential amino acids (**Table 4**; **Supplementary Table 8**). Small but significant median heritability
200 differences were observed among the different classes of lipids (**Figure 3**). For lipid classes the $h^2_{Metabolite\text{-}}$
201 $_{hits}$ estimates differed significantly ($F(8, 300) = 8.47$; FDR-adjusted p-value = 0.004; **Supplementary Table**
202 **6**).

203 Finally, we explored whether heritability of phosphatidylcholines and TGs increases with a larger
204 number of carbon atoms and/or double bonds in their fatty acyl side chains. To this end we employed
205 both uni- and multivariate mixed-effect meta-regression models separately for the TGs, diacyl

206 phosphatidylcholines (PCaa) and acyl-alkyl phosphatidylcholines (PCae; see **Methods**). The platform
207 specific heritability estimates for each of these lipid species are depicted in **Supplementary Figure 2**.
208 Variation in the number of carbon atoms and double bonds was significantly associated with $h^2_{Metabolite-}$
209 $_{hits}$ estimates for PCaa's ($F(3, 52) = 7.05$; FDR-adjusted p-value = 0.009) and PCae's ($F(3, 45) = 3.41$; FDR-
210 adjusted p-value = 0.05; **Supplementary Table 6**). Phosphatidylcholines with a larger number of carbon
211 atoms showed lower heritability estimates and phosphatidylcholines with a larger number of double
212 bonds had higher heritability estimates (**Supplementary Table 6**). The differences among the
213 phosphatidylcholines with a variable number of carbon atoms and/or double bonds may have
214 contributed to differential h^2_{Class} estimates. Univariate models confirmed the results for the number of
215 double bonds in PCaa's and PCae, though they were not significant after correction for multiple testing
216 (**Supplementary Table 8**).

217 Discussion

218 We carried out a comprehensive assessment of GWA-metabolomics studies, and created a repository of
219 all studies reporting on associations of SNPs and blood metabolites in European ancestry samples. We
220 curated 241,965 genome-wide metabolite associations and we classified the associated metabolites into
221 super classes, classes and sub-classes. The complete overview of all blood metabolite-SNP associations is
222 provided in **Supplementary Data 1** (http://bbmri.researchlumc.nl/atlas/#data), with the complete list of
223 references in **Supplementary Note 1**. The information from the repository was used to construct GRMs,
224 which served to identify genetic variance components in the analysis of 369 metabolites. The metabolite
225 data in our study came from a large cohort of twin-families (N = 5,117 clustered in 2,445 families)
226 measured on four metabolomics platforms. We focused on two metabolite super classes. By mapping all
227 metabolites to the Human Metabolome Database (HMDB)[2–5] we were able to classify both the
228 measured metabolites and all previously published metabolites as either lipids or organic acids. In the

229 current study, we sought to elucidate the contribution of known metabolite loci, based on a decade of
230 GWA and (exome-) sequencing studies, to metabolite levels ($h^2_{Metabolite\text{-}hits}$). A unique feature of our study
231 was the ability to disentangle the role of class-specific ($h^2_{Class\text{-}hits}$) and non-class ($h^2_{Notclass\text{-}hits}$) metabolite
232 loci on heritability differences among metabolite classes and lipid species.

233 To evaluate differences among metabolite classes and lipid species in the estimates for h^2_{total}, we
234 applied multivariate mixed-effect meta-regression models to the estimates of $h^2_{Metabolite\text{-}hits}$, $h^2_{Class\text{-}hits}$, and
235 $h^2_{Notclass\text{-}hits}$. We observed no significant differences in h^2_{total} estimates among the metabolite classes.
236 Consistent with a previous twin-family study[13], none of the heritability estimates differed significantly
237 among essential and non-essential amino acids. We observed significant $h^2_{Metabolite\text{-}hits}$ differences among
238 the different classes of organic acids. Keto acids had significantly lower $h^2_{Metabolite\text{-}hits}$ estimates as
239 compared with carboxylic acids. Class-specific metabolite loci heritability estimates for fatty acyls,
240 lipoproteins and steroids were significantly higher. Similarly, significant heterogeneity in lipid class
241 heritability, with lower h^2_{total} and h^2_{SNP} for phospholipids than for sphingolipids or glycerolipids has been
242 reported[16,18,32]. Lastly, we assessed whether heritability increases with added complexity in lipid
243 species[14,19]. We found that this was the case with respect to $h^2_{Metabolite\text{-}hits}$ estimates in more complex
244 diacyl and acyl-alkyl phosphatidylcholines, but not for more complex TGs. Previous research reported
245 significant higher h^2_{SNP} estimates in polyunsaturated fatty acid containing lipids[18]. Furthermore, loci
246 associated with traditional lipid measures explained 2% to 21% of the variance in lipid levels[18]. Together
247 these results suggest that higher heritability in phosphatidylcholines is driven by a lower number of
248 carbon atoms and higher number of double bonds, e.g., a larger degree of unsaturation.

249 Evaluating the mean heritability differences among lipids and organic acids, it appears that lipids
250 have higher h^2_{total}, $h^2_{Class\text{-}hits}$ and $h^2_{Metabolite\text{-}hits}$ estimates than organic acids (**Table 3**). Previous twin-family
251 studies indicates that the heritability difference among lipids and organic acid is rarely investigated[12–15].

252 This is possibly because most metabolomics platforms focus mainly on either lipids or organic acids.
253 Lipid metabolite classes tend to be very well represented on metabolomics platforms, whereas organic
254 acids are unrepresented, and as a consequence, the analysis to obtain $h^2_{Class\text{-}hits}$ and $h^2_{Metabolite\text{-}hits}$
255 estimates of the organic acids will be underpowered due to this imbalance.

256 Limitations

257 The current study has several limitations. First, the extent to which our findings generalize to
258 populations of non-European ancestry is unknown. Loci of common human metabolism pathways are
259 most likely to replicate over ethnicities[33]. Second, estimates of the total variance explained may show
260 upward bias when based on data from closely related individuals (e.g., first cousins or closer)[22,23]. This
261 bias is caused by the influence of shared environmental influences, epistatic interactions, or
262 dominance[22,23]. While the results of the current study may suffer of such biases by the inclusion of twins,
263 siblings and parents, the sample also includes many unrelated individuals which will reduce the possible
264 bias (**Supplementary Figure 3**).

265 Kettunen et al. (2012) investigated 217 metabolites of the Nightingale Health ^{1}H-NMR platform
266 in a classical twin design and reported dominance effects for 6.45% of the metabolites[34]. Tsepsilov et al.
267 (2015) performed GWA study targeting non-additive genetic effects and concluded that most genetic
268 effects on metabolite levels and ratios were in fact additive[35]. Together, these studies suggested that the
269 bias due to dominance effects on metabolite levels will be minor.

270 Relatively few twin-family studies explicitly investigated the role of shared environmental
271 influences on metabolite levels. Overall, shared environmental influences are reported for a small
272 number of metabolites (e.g., 14.3% of all Nightingale Health ^{1}H-NMR metabolites[34]) and the influence of
273 the shared environment is small-to-moderate (platform and metabolite class-dependent averages range
274 from 0.03 to 0.45[9,16,36–38] with larger estimates deriving from small studies). For studies including parents

275 and offspring, or adult twin and siblings pairs the question arises which effects are captured by the
276 shared environment. Are these the lasting influences of the environment offspring shared with their
277 parents and with each other before they started living independently? Additional research is necessary
278 to elucidate the role of the shared environment on metabolite levels[22].

279 Third, standard errors of h^2_{SNP} estimates were high. While we have included all h^2_{SNP} estimates in the
280 supplements, we stress that the primary goal of our paper was to investigate the contribution of known
281 metabolite loci in an independent sample rather than obtaining the h^2_{SNP} estimates for metabolites.

282 Finally, the estimates for $h^2_{metabolite\text{-}hits}$ are based on SNPs of 40 different studies from a decade of
283 GWA and (exome-) sequencing studies. The sample size, and therefore the power, of these studies vary,
284 with some studies conducted with as few as 211 individuals while others included over 24,000
285 individuals (**Supplementary Note 1**). For underrepresented metabolites the low power may result in
286 downward biased heritability estimates. However, leveraging information from a decade of research in
287 40 studies and extracting loci for metabolite classes across multiple studies, the number of such
288 metabolites is not large. New[32,39–41] and future studies will increase the number of variants identified as
289 metabolite loci. The investment in UK Biobank[42] is expected to dramatically increase sample sizes for
290 large-scale genomic investigations of the human metabolome and subsequently the number of
291 metabolite loci.

292 Future directives and conclusions

293 Mendelian Randomization may benefit from the comprehensive overview of metabolite loci that we
294 identified. The identified loci can serve as instruments in metabolome-wide Mendelian Randomization
295 studies of complex traits. In addition, our work offers valuable insights into the role of common genetic
296 variants in class specific heritability differences among metabolite classes and lipids species. Further
297 research is required to elucidate the contribution of rare genetic variants to metabolite levels, and

298 differences in the contribution of rare genetic variants among metabolite classes. A reasonable
299 approach would be to carry out a similar study in a large sample of whole-genome sequencing (WGS)
300 data. Such an approach, using MAF- and LD-stratified GREML analysis[43], identified additional variance
301 due to rare variants for height and BMI[44].

302 In conclusion, we contributed to our understanding of the genetic architecture of fasting blood
303 metabolite levels, and of differences in the genetic architecture among metabolite classes. Extending
304 the GREML framework with the inclusion of known metabolite loci allowed us to simultaneously
305 estimate h^2_{total}, and $h^2_{metabolite\text{-}hits}$ (which consists of $h^2_{Class\text{-}hits}$ and $h^2_{Notclass\text{-}hits}$) for 361 metabolites.
306 Significant differences in $h^2_{Metabolite\text{-}hits}$ estimates were observed among different classes of lipids and
307 organic acids and for more complex diacyl and acyl-alkyl phosphatidylcholines. Future studies should
308 address the proportion of metabolite variation influenced by heritable and non-heritable lifestyle
309 factors, as this will facilitate the development of personalized disease prevention and treatment of
310 complex disorders.

311 # Methods

312 ## Participants

313 At the Netherlands Twin Register (NTR)[45] metabolomics data for twins and family members as measured
314 in blood samples were available for 6,011 individuals of whom 5,667 were genotyped. The blood
315 samples for the four metabolomics experiments described in this study were mainly collected in
316 participants of the NTR biobank project[28,46]. Blood samples were collected after a minimum of two hours
317 of fasting (1.3%), with the majority of the samples collected after overnight fasting (98.7%). Fertile
318 women were bled in their pill-free week or on day 2-4 of their menstrual cycle. For the current paper,
319 we excluded participants who were not of European ancestry, who were on lipid-lowering medication at

320 the time of blood draw, and who failed to adhere to the fasting protocol. The exact number of
321 exclusions per dataset is listed in **Supplementary Table 9**. After completing the preprocessing of the
322 metabolomics data, the separate subsets (e.g., different collection and measurement waves; see
323 **Supplementary Table 9**) of each platform were merged into a single per platform dataset, retaining a
324 single (randomly chosen) observation per platform when multiple observations were available.
325 **Supplementary Table 10** gives an overview of the overlap in participants among the different platforms,
326 with the overlap among each metabolite that survived quality control (QC) for all four platforms
327 available in **Supplementary Table 11**. The final number of participants included in the study was 5,117,
328 with platform specific sample size ranging from 1,448 to 4,227 individuals clustered in 946 to 2,179
329 families. Characteristics for the individuals can be found in **Table 2**. **Supplementary Figure 3** depicts the
330 distribution of the relatedness in the sample. Informed consent was obtained from all participants.
331 Projects were approved by the Central Ethics Committee on Research Involving Human Subjects of the
332 VU University Medical Centre, Amsterdam, an Institutional Review Board certified by the U.S. Office of
333 Human Research Protections (IRB number IRB00002991 under Federal-wide Assurance- FWA00017598;
334 IRB/institute codes, NTR 03-180 and EMIF-AD 2014.210).

335 Metabolite profiling

336 Nightingale Health ^{1}H-NMR platform

337 Metabolic biomarkers were quantified from plasma samples using high-throughput proton nuclear
338 magnetic resonance spectroscopy (^{1}H-NMR) metabolomics (Nightingale Health Ltd, Helsinki, Finland;
339 formerly Brainshake Ltd.). This method provides simultaneous quantification of routine lipids,
340 lipoprotein subclass profiling with lipid concentrations within 14 subclasses, fatty acid composition, and
341 various low-molecular weight metabolites including amino acids, ketone bodies and glycolysis-related

342 metabolites in molar concentration units. Details of the experimentation and epidemiological
343 applications of the NMR metabolomics platform have been reviewed previously[47,48].

344 **UPLC-MS lipidomics platform**

345 Plasma lipid profiling was performed at the division of Analytical Biosciences at the Leiden Academic
346 Center for Drug Research at Leiden University/Netherlands Metabolomics Centre. The lipids were
347 analyzed with an Ultra-High-Performance Liquid Chromatograph directly coupled to an Electrospray
348 Ionization Quadruple Time-of-Flight high resolution mass spectrometer (UPLC-ESI-Q-TOF; Agilent 6530,
349 San Jose, CA, USA) that uses reference mass correction. For liquid chromatographic separation a
350 ACQUITY UPLC HSS T3 column (1.8µm, 2.1 ∗ 100mm) was used with a flow of 0.4 ml/min over a 16
351 minute gradient. Lipid detection was done using a full scan in the positive ion mode. The raw MS data
352 were pre-processed using Agilent MassHunter Quantitative Analysis software (Agilent, Version B.04.00).
353 Detailed descriptions of lipid profiling and quantification have been described previously[49,50].

354 **Leiden ^{1}H-NMR platform (for small metabolites)**

355 The Leiden ^{1}H-NMR spectroscopy experiment of EDTA-plasma samples used a 600 MHz Bruker Advance
356 II spectrometer (Bruker BioSpin, Karlsruhe, Germany). The peak deconvolution method used for this
357 platform has been previously described[51].

358 **Biocrates Absolute-IDQ™ p150 platform**

359 The Biocrates Absolute-IDQ™ p150 (Biocrates Life Sciences AG, Innsbruck, Austria) metabolomics
360 platform on serum samples was analyzed at the Metabolomics Facility of the Genome Analysis Centre at
361 the Helmholtz Centre in Munich, Germany. This platform utilizes flow injection analysis coupled to
362 tandem mass spectrometry (MS/MS) and has been described in detail elsewhere[7,52,53].

363 ## Metabolomics data preprocessing

364 Preprocessing of the metabolomics data was done separately for each of the platforms and each
365 measurement batch. Metabolites were excluded from analysis when the mean coefficient of variation
366 exceeded 25% and the missing rate exceeded 5%. Metabolite measurements were set to missing if they
367 were below the lower limit of detection or quantification or could be classified as an outlier (five
368 standard deviations greater or smaller than the mean). Metabolite measurements, which were set to
369 missing because they fell below the limit of detection/quantification were imputed with half of the value
370 of this limit, or when this limit was unknown with half of the lowest observed level for this metabolite.
371 All remaining missing values were imputed using multivariate imputation by chained equations
372 ('mice')[54]. On average, 9 values were imputed for each metabolite (SD = 12; range: 1-151). Data for each
373 metabolite on both ^{1}H-NMR platforms were normalized by inverse normal rank transformation[51,55],
374 while the imputed values of the Biocrates metabolomics platform and the UPLC-MS lipidomics platform
375 were normalized by natural logarithm transformation[14,56], conform previous normalization strategies
376 applied to the data obtained using these platforms. The complete lists with full names of all detected
377 metabolites that survived QC and preprocessing for all platforms can be found in **Supplementary Table**
378 **1**, these tables also include the quartile values of the untransformed metabolites.

379 ## Genotyping, imputation and ancestry outlier detection

380 Genotype information was available for 21,001 NTR participants from 6 different genotyping arrays
381 (Affymetrix 6.0 [*N* = 8,640], Perlegen-Affymetrix [*N* = 1,238], Illumina Human Quad Bead 660 [*N* = 1,439],
382 Affymetrix Axiom [*N* = 3,144], Illumnia GSA [*N* = 5,938] and Illumina Omni Express 1M [*N* =238]), as well as
383 sequence data from the Netherlands reference genome project GONL (BGI full sequence at 12x (*N* = 364)[57].
384 For each genotyping array samples were removed if they had a genotype call rate above 90%, gender-
385 mismatch occurred or if heterozygosity (Plink F statistic) fell outside the range of -0.10 – 0.10. SNPs were

386 removed if they were palindromic AT/GC SNPs with a minor allele frequency (MAF) range between 0.4 and
387 0.5, if the MAF was below 0.01, if Hardy Weinberg Equilibrium (HWE) had $p < 10^{-5}$, and if the number of
388 Mendelian errors was greater than 20 and the genotype call rate was < 0.95. After QC the six genotyping
389 arrays were aligned to the GONL reference set (V4) and SNPs were removed if the alleles mismatched with
390 this reference panel or the allele frequency different more than 0.10 between the genotyping array and this
391 reference set.

392 The data from the six genotyping chips were subsequently merged into a single dataset (1,781,526
393 SNPs). Identity-by-decent (IBD) was estimated with PLINK[58] and KING[59] for all individual pairs based on the
394 ~10.6K SNPs in common across the arrays. Next IBD was compared to expected family relations and
395 individuals were removed in the event of a mismatch. Prior to imputation to the GONL reference data[60,61] the
396 duplicate monozygotic pairs (N = 3,032) or trios (N = 7) and NTR GONL samples (N = 364) were removed and
397 the data was cross-array phased using MACH-ADMIX[62]. Post-imputation the NTR GONL samples and the
398 duplicated MZ pairs and trios were re-turned to the dataset. Filtering of the imputed dataset included the
399 removal of SNPs that were significantly associated with a single genotyping chip ($p < 10^{-5}$), had HWE $p < 10^{-5}$,
400 the Mendelian error rate > mean + 3 SD, or imputation quality (R^2) below 0.90. The final cross-platform
401 imputed dataset included 1,314,639 SNPs, including 20,792 SNPs on the X-chromosome.

402 The cross-platform imputed data was aligned with PERL based "HRC or 1000G Imputation preparation
403 and checking" tool (version 4.2.5; https://www.well.ox.ac.uk/~wrayner/tools). The remaining 1,302481 SNPs
404 were phased with EAGLE[63] for the autosomes, and SHAPEIT[64] for chromosome X and then imputed to 1000
405 Genomes Phase 3 (1000GP3 version 5)[65] on the Michigan Imputation server using Minimac3 following the
406 standard imputation procedures of the server[66]. Principal Component Analysis (PCA) was used to project the
407 first 10 PCs of the 1000 genomes references set population on the NTR cross-platform imputed data using
408 SMARTPCA[67]. Ancestry outliers (non-Dutch ancestry; N = 1,823) were defined as individuals with PC values

409 outside the European/British population range[68]. After ancestry outlier removal the first 10 PCs were
410 recalculated.

411 Curation of metabolite loci

412 In October 2018 PubMed and Google Scholar were searched to identify published GWA and (exome-)
413 sequencing studies on metabolomics or fatty acid metabolism in blood samples using ^{1}H-NMR, mass
414 spectrometry or gas chromatography-based methods. In the period of November 2008 to October 2018
415 40 GWA or (exome-) sequencing studies on blood metabolomics in European samples were published
416 (**Supplementary Note 1**). The genome-wide significant ($p < 5x10^{-8}$) metabolite-SNP associations of all
417 studies were extracted, including only those observations for autosomal SNPs and reporting SNP effect
418 sizes and p-values based on the summary statistics excluding NTR samples[55,56]. In the 40 studies, 242,580
419 metabolite-SNP or metabolite ratio-SNP associations were reported. These associations included 1,804
420 unique metabolites or ratios and 49,231 unique SNPs (**Supplementary Data 1**). For all metabolites their
421 Human Metabolome Database (HMDB)[3–5], PubChem[69], Chemical Entities of Biological Interest (ChEBI)[70]
422 and International Chemical Identifier (InChiKey)[71] identifiers were retrieved. Information with regards to
423 the 'super class', 'class' and 'subclass' of metabolites was extracted from HMDB. If no HMDB identifier
424 was available and categorization information could not be extracted, 'super class', 'class' and 'subclass'
425 were provided based on expert opinion. Excluding the ratios and unidentified metabolites, 953
426 metabolites were classified into 12 'super classes', 43 'classes' or 77 'subclasses' (**Supplementary Data**
427 **1**). Based on the metabolite identifiers we also extracted the *log(S)* value for each metabolite to assess
428 the hydrophobicity of the metabolites. The *log(S)* value represents the log of the partition coefficient
429 between 1-octanol and water, two fluids that hardly mix. The partition coefficient is the ratio of
430 concentrations in water and in octanol when a substance is added to an octanol-water mixture and
431 hence indicates the hydrophobicity of a compound. Thus, we classified a metabolite as hydrophobic if it
432 is more hydrophobic than 1-octanol, and as hydrophilic otherwise (**Supplementary Data 1**).

433 The rsIDs or chromosome-base pair positions of the 49,231 unique SNPs were reported by
434 different genome builds or dbSNP maps[72], therefore we lifted all SNPs to HG19 build 37[73], after which
435 43,830 unique SNPs remained (**Supplementary Figure 1**; **Supplementary Data 1**). All bi-allelic metabolite
436 SNPs were extracted from our 1000GP3 data, which excluded 295 tri-allelic SNPs, and 4,256 SNPs that
437 could not be retrieved from 1000GP3. Next, MAF > 1% (2,067 SNPs removed), $R^2 > 0.70$ (2,002 SNPs) and
438 HWE $P < 10^{-4}$ (72 SNPs) filtering was performed, resulting in 35,138 metabolite SNPs for NTR participants
439 (**Supplementary Figure 1**). Next, we created two 'super class'-specific lists of metabolite loci and two
440 'not-superclass' lists of metabolite loci. To create a list of loci associated with the 652 unique
441 metabolites classified as 'lipids and lipid-like molecules' (e.g., lipids), we clumped (PLINK version 1.9) all
442 112,760 lipid-SNP associations using an LD-threshold (r^2) of 0.10 in a 500kb radius in 2,500 unrelated
443 individuals (**Supplementary Figure 1**). Clumping identified 482 lead SNPs, or loci for lipids. An additional
444 12,169 SNPs were identified as LD-proxies for the lipid-loci (**Supplementary Figure 1**). To obtain the 'not-
445 superclass' list of lipid loci the 12,651 lipid loci and proxies were removed from the list of all metabolite-
446 SNP associations and the resulting list was clumped to obtain the 598 'non-superclass' loci
447 (**Supplementary Figure 1**). The same clumping procedure was applied to the 26,352 organic acid-SNP
448 associations, identifying 398 organic acids loci, 10,781 organic acid LD-proxies and 687 'non-superclass'
449 loci (**Supplementary Figure 1**).

450 Construction of genetic relationship matrices

451 In total six weighted genetic relationship matrixes (GRMs) were constructed, which were corrected for
452 uneven and long-range LD between the SNPs (LDAK version 4.9[29,30]). In **Supplementary Note 3** the use of
453 weighted versus unweighted GRMs is compared using simulations. Two of the GRMs used the cross-platform
454 imputed dataset as backbone and the other four GRMs were based on SNPs extracted from the 1000GP3
455 imputed data. Before calculating the first GRM, the autosomal SNPs of the cross-platform imputed dataset
456 were filtered on MAF (<1%) and all lipid and organic acid loci, their LD-proxies and 50kb surrounding both

457 types of SNPs were removed (see **curation of metabolite loci**; **Supplementary Figure 1**). The LDAK GRM was
458 created after removal of the 50kb surrounding the lipid and organic acid loci and their LD-proxies (as obtained
459 by the clumping procedure as described above) and included 434,216 SNPs (**Supplementary Figure 1**). The
460 *V(G1)* variance component in the genomic relatedness matrix residual maximum likelihood (GREML) analyses
461 is based on this GRM (see **heritability analyses**; **Figure 1**). The *V(G2)* variance component in the GREML
462 analyses is based on the LDAK GRM including all autosomal SNPs with a MAF greater than 1% included on the
463 cross-platform imputed dataset (447,794 SNPs), where ancestry outliers were removed, and genome sharing
464 was set to zero for all individual pairs sharing less than 0.05 of their genome[22] (**Figure 1**). Depending on the
465 metabolite the *V(G3)* variance component in the GREML analyses was either based on an LDAK GRM of the
466 1000GP3 extracted lipid loci (479 SNPs) or the organic acid loci (397 SNPs), as obtained after the clumping
467 procedure as described above (**Supplementary Figure 1**; **Figure 1**). Finally, depending on the metabolite
468 either the 'not-lipid' LDAK GRM (596 SNPs) or the 'not-organic acid' LDAK GRM (683 SNPs) provided the *V(G4)*
469 variance component in the GREML analyses (**Supplementary Figure 1**; **Figure 1**). The not-class metabolite loci
470 on which the LDAK GRMs were build were obtained by the clumping procedure as described above
471 (**Supplementary Figure 1**). **Supplementary Data 1** indicates for each listed SNP if it was included in any of the
472 class-specific or not-class LDAK GRMs.

473 Statistical analyses

474 Heritability analyses

475 Mixed linear models[22], implemented in the genome-wide complex trait analysis (GCTA) software
476 package (version 1.91.7)[21], were applied to compare three models including a variable number of
477 covariates. **Supplementary Table 12** gives the three different models, full descriptions of the covariates
478 and model comparison have been given in **Supplementary Note 4**. The most parsimonious model was
479 chosen for further analyses (full results in **Supplementary Table 13**). This final model included the first

480 10 genetic PCs for the Dutch population, genotyping chip, sex and age at blood draw as covariates. For
481 metabolites of the Nightingale Health ^{1}H-NMR and Biocrates platform, measurement batch was included
482 as covariate.

483 The final four-variance component model, including four GRMs, allows for the estimation of the
484 proportion of variation explained by superclass-specific significant metabolite loci and non-superclass
485 significant metabolite loci. The first two variance components in the 4-variance component model
486 (**Figure 1**), *V(G1)* and *V(G2)* allow for the estimation of the additive genetic variance effects captured by
487 genome-wide SNPs (h^2_g) and the additive genetic effects associated with pedigree (h^2_{ped})[22,74], and *V(G3)*
488 and *V(G4)* capture the additive genetic effect associated with class-specific ($h^2_{class\text{-}hits}$) and not-class
489 ($h^2_{notclass\text{-}hits}$) metabolite loci. Based on the 4-variance component model, three additional heritability
490 estimates can be calculated: the total variance explained by significant metabolite loci ($h^2_{Metabolite\text{-}hits}$)
491 consists of the sum of $\frac{V(G3)}{Vp}$ and $\frac{V(G4)}{Vp}$, where *Vp* is the phenotypic variance, h^2_{SNP} is defined as the sum of
492 $\frac{V(G1)}{Vp}$, $\frac{V(G3)}{Vp}$ and $\frac{V(G4)}{Vp}$, and the total variance explained (h^2_{total}) is defined as the sum of $\frac{V(G1)}{Vp}$, $\frac{V(G2)}{Vp}$, $\frac{V(G3)}{Vp}$
493 and $\frac{V(G4)}{Vp}$ (**Figure 1**). We note that the total variance explained by genetic factors may also include
494 influences of the shared environment, dominance and epistasis, which may result in upward bias of the
495 h^2_{total} estimates[22,23]. This bias is expected to arise by the presence of closely related participants, who
496 may share these effects, in addition to the additive genetic effects. To calculate the standard errors
497 (s.e.'s) for the composite variance estimates, we have randomly sampled 10,000 new variances from the
498 parameter variance-covariance matrices of the *V(G1), V(G3)* and *V(G4)* GRMs for each metabolite.
499 Random sampling was performed in R by creating 10,000 multivariate normal distributions (mvrnorm
500 function in MASS package version 7.3-50[75]) based on the original means and variance/covariance
501 matrices. The s.e.'s of the specific ratio of interest were then based on the standard deviation of the
502 ratio of interest across 10,000 samples. The four-variance component models included variance

503 components that were not constrained to be positive, thus allowing for negative h^2_{SNP} and $h^2_{Metabolite\text{-}hits}$

504 estimates. All four-variance component models applied the --reml-bendV flag where necessary to invert

505 the variance-covariance matrix *V* if *V* was not positive definite, which may occur when variance

506 components are negative[76]. Finally, we calculated the log likelihood of a reduced model with either

507 *V(G3)*, *V(G4)* or both dropped from the full model and calculated the LRT and p-value (**Supplementary**

508 **Table 3**).

509 ## Mixed-effect meta-regression analyses

510 To investigate differences in heritability estimates among metabolites of different classes we applied

511 mixed-effect meta-regression models as implemented in the 'metafor' package (version 2.0-0) in R

512 (version 3.5.1)[77]. Here we tested for the moderation of heritability estimates by metabolite class and

513 metabolomics platform on all 361 successfully analyzed metabolites. We included a matrix combining

514 the phenotypic correlations (**Supplementary Table 14**) and the sample overlap (**Supplementary Table**

515 **11**) between the metabolites as random factor to correct for dependence among the metabolites and

516 participants. This matrix includes the sample size of the metabolite on the diagonal, with the off-

517 diagonal computed by $\frac{N_{1,2}}{\sqrt{n_1 * n_2}} * r$ (**Supplementary Table 15**), where $N_{1,2}$ is the sample overlap between

518 the metabolites, n_1 is the sample size of metabolite one, n_2 is the sample size of metabolite two and *r* is

519 the phenotypic (Spearman's rho) correlation between the metabolites. In all mixed-effect meta-

520 regression analyses we obtained the robust estimates based on a sandwich-type estimator, clustered by

521 the metabolites included in the models to correct for the sample overlap among the different

522 metabolites[78]. First, we used multivariate mixed-effect meta-regression models to simultaneously

523 estimate the effect of metabolite class and metabolomics platform on the h^2_{total}, h^2_{SNP} and the $h^2_{Metabolite\text{-}}$

524 $_{hits}$, as well as the $h^2_{Class\text{-}hits}$ and $h^2_{Notclass\text{-}hits}$ estimates. Subsequently, to separately assess the effect of the

525 number of carbon atoms or double bonds in the fatty acyls chains of phosphatidylcholines and

526 triglycerides univariate models were fitted, as follow-up. To account for multiple testing the p-values
527 were adjusted with the with the False Discovery Rate (FDR)[79] using the 'p.adjust' function in R. Multiple
528 testing correction was done separately for the univariate and the multivariate models.

529 Data availability

530 The curated list of all published metabolite-SNP associations is included in **Supplementary Data 1** and is
531 publicly available through the BBMRI – omics atlas (http://bbmri.researchlumc.nl/atlas/#data). All
532 information on the metabolites in this study are in **Supplementary Table 1**; with full summary statistics
533 for the four-variance component models included in **Supplementary Table 3**. The Nightingale Health
534 metabolomics data may be requested through BBMRI-NL (https://www.bbmri.nl/Omics-metabolomics).
535 All (other) data may be accessed, upon approval of the data access committee, through the Netherlands
536 Twin Register (ntr.fgb@vu.nl). A reporting summary for this Article is available as Supplementary
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Author contributions

Nightingale Health metabolomics data: HES, MBeekman, PES and CMvD. Leiden ^{1}H-NMR metabolomics data: KWvD and AV. UPLC-MS lipidomics data: ACH and TH. EMIF-AD data: AdB and PJV. Genotype data: JJH, AA and IOF. NTR Biobank data: GW and EJCdG. Metabolomics pre-processing: RP, HHMD and FAH. Statistical analyses: FAH and MGN. Wrote the paper: FAH, JvD, MBartels, MGN and DIB. All authors critically read and commented on the manuscript.

569 ## Competing interests statement

570 The authors declare no competing financial interests.

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Figures

Figure 1. Overview of the 4-variance component models, including the GRMs underlying each variant component and all heritability estimates obtained from the models.

Overview of the SNP-filtering and GRM construction can be found in **Supplementary Figure 1** and is explained in details in the **Methods**. This figure describes which GRMs (black boxes) are used to calculate which variance components (orange boxes) by drawing black arrows from the GRMs to the variance components. The variance components give rise to the four different heritability estimates: h^2_{ped}, h^2_{g}, $h^2_{Class\text{-}hits}$, and $h^2_{Notclass\text{-}hits}$ (see **Methods**). The orange arrows indicate how the various variance components are summed to obtain estimates for $h^2_{metabolite\text{-}hits}$, h^2_{SNP} and h^2_{total} (see **Methods**).

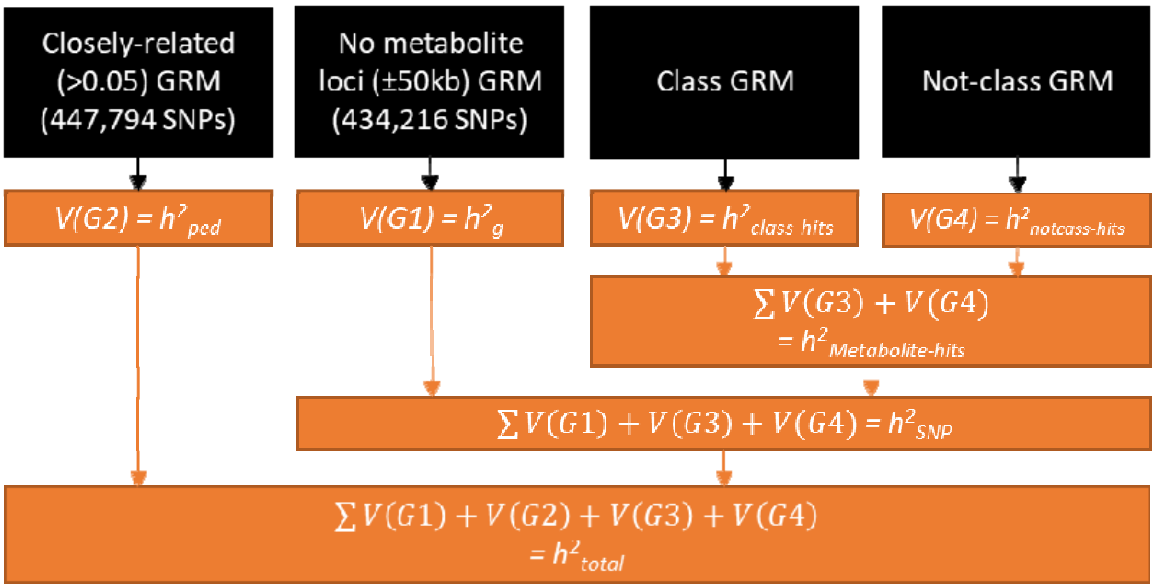


Figure 2. Heritability of all 52 carboxylic acids and derivatives successfully analyzed across all four metabolomics platforms by class.

Box- and dotplots of the h^2_{total}, and $h^2_{Metabolite\text{-}hits}$ for all 52 successfully analyzed 'carboxylic acids and derivatives' by class. The left-hand side of the figure is a close-up of the -0.08 – 0.15 part of the heritability range, focusing on the $h^2_{Class\text{-}hits}$ and $h^2_{Notclass\text{-}hits}$ estimates. The boxes denote the 25th and 75th percentile (bottom and top of box), and median value (horizontal band inside box). The whiskers indicate the values observed within up to 1.5 times the interquartile range above and below the box. **Supplementary Table 3** provides the estimates for each of the individual metabolites.

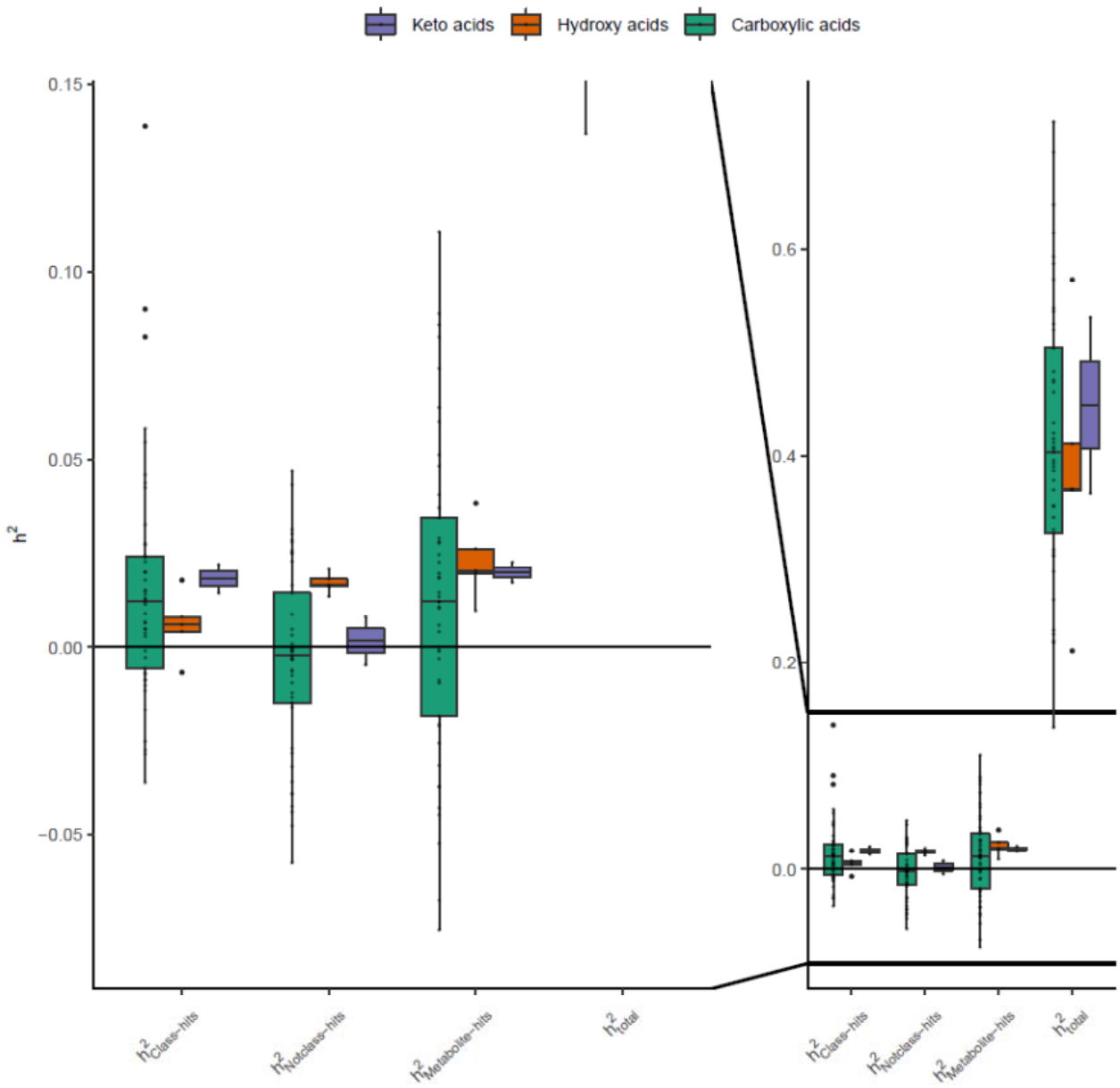
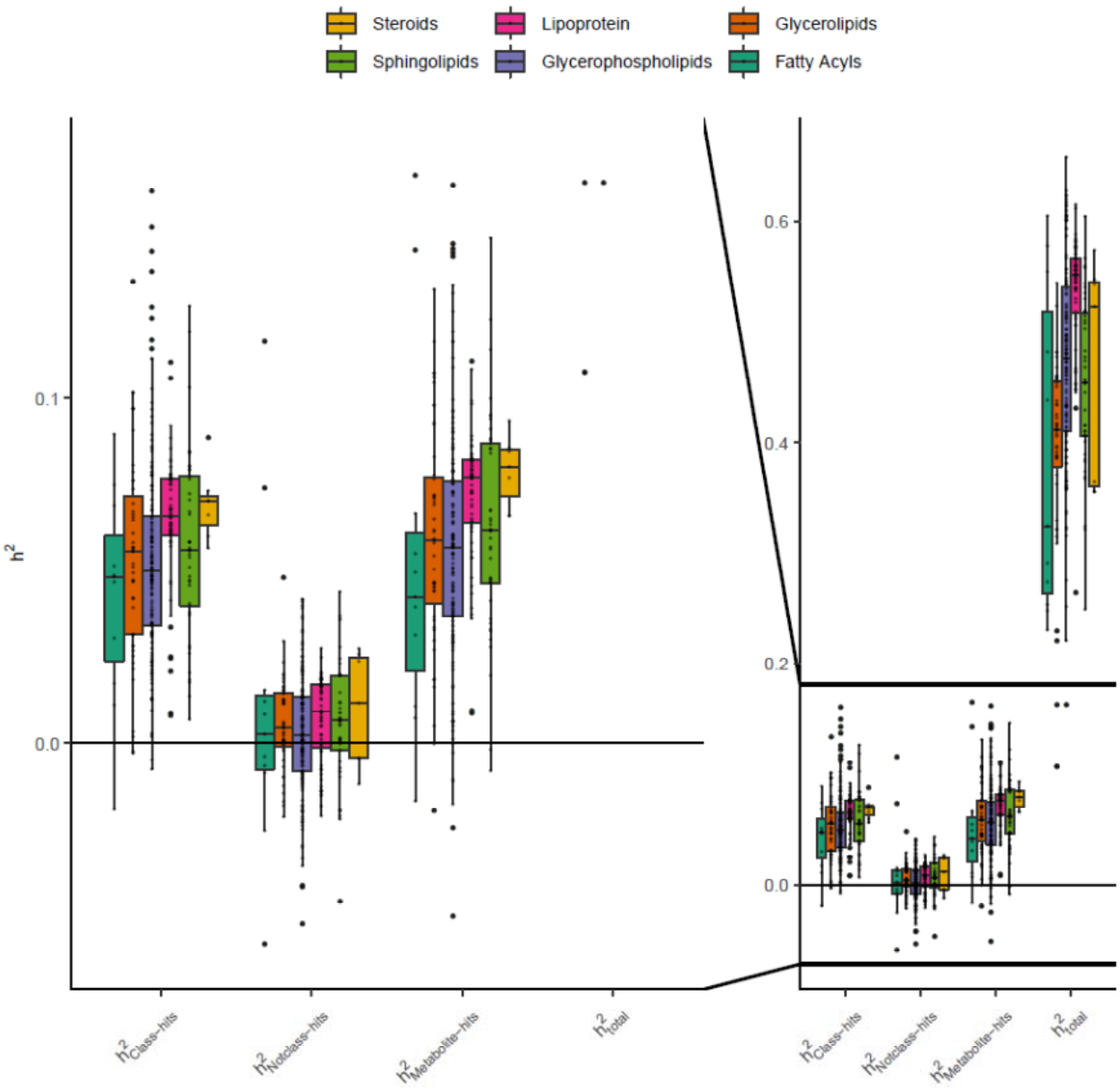

Keto acids
Hydroxy acids
Carboxylic acids
0.15
0.10
0.05
0.00
−0.05
h2
0.6
0.4
0.2
0.0
h2Class−hits
h2Noclass−hits
h2Metabolite−hits
h2total

Figure 3. Heritability of all 309 lipids successfully analyzed across all four metabolomics platforms by class.

Box- and dotplots of the h^2_{total}, and $h^2_{Metabolite\text{-}hits}$ for all 309 successfully analyzed lipids by class. The left-hand side of the figure is a close-up of the -0.06 – 0.17 part of the heritability range, focusing on the $h^2_{Class\text{-}hits}$ and $h^2_{Notclass\text{-}hits}$ estimates. The boxes denote the 25th and 75th percentile (bottom and top of box), and median value (horizontal band inside box). The whiskers indicate the values observed within up to 1.5 times the interquartile range above and below the box. **Supplementary Table 3** provides the estimates for each of the individual metabolites.


Steroids
Lipoprotein
Glycerolipids
Sphingolipids
Glycerophospholipids
Fatty Acyls
h2
0.1
0.0
0.6
0.4
0.2
0.0
h2Class-hits
h2Notclass-hits
h2Metabolite-hits
h2total

Tables

Table 1. Overview of the number of unique metabolites, for which significant SNP-metabolite associations have been published, per Human Metabolome Database[3–5] 'super class'.

See **Supplementary Data 1** for an overview of the exact metabolites classified per 'super class', 'class' and 'subclass', as well as the SNPs associated with each metabolite.

Super class	Number of unique metabolites
Lipids and lipid-like molecules (e.g., lipids)	662
Organic acids and derivatives (e.g., organic acids)	182
Organoheterocyclic compounds	45
Organic oxygen compounds	19
Nucleosides, nucleotides, and analogues	12
Benzenoids	12
Organic nitrogen compounds	11
Phenylpropanoids and polyketides	4
Proteins	3
Organic compounds	1
Trichlorophenols	1
Organooxygen compounds	1

Table 2. Participant characteristics after preprocessing per metabolomics platform.

This table gives an overview of the number of individuals (N) per platform, specifies the number of families these individuals belong to and the percentage of females and twins in each dataset. In addition, for each platform the mean and standard deviation (SD) of the age at blood draw in years, the body-mass-index (BMI), the cholesterol level in mmol/l, the low-density lipoprotein cholesterol (LDL) levels in mmol/l and the high-density lipoprotein cholesterol (HDL) levels in mmol/l are given.

Metabolomics platform	N	N families	Age* (mean ± SD)	Female (%)	Twins (%)	BMI (mean ± SD)	Cholesterol$ (mean ± SD)	LDL$ (mean ± SD)	HDL$ (mean ± SD)
All Participants	5,117	2,445	42.1 ± 14.2	62.8%	63.4%	24.8 ± 4.1	4.9 ± 1.2	3.0 ± 1.0	1.7 ± 1.0
Nightingale Health ^{1}H-NMR	4,227	2,179	40.7 ± 13.7	67.3%	69.7%	24.6 ± 4.0	4.9 ± 1.2	3.0 ± 1.0	1.7 ± 1.0
UPLC-MS Lipidomics	2,324	1,251	39.0 ± 12.9	66.6%	89.2%	24.4 ± 4.1	5.0 ± 1.0	3.0 ± 0.9	1.4 ± 0.4
Leiden ^{1}H-NMR	2,324	1,323	37.6 ± 12.5	67.0%	89.0%	24.2 ± 4.1	4.6 ± 1.3	2.7 ± 1.0	2.0 ± 1.4
Biocrates	1,448	946	45.7 ± 15.3	43.8%	39.6%	25.2 ± 3.9	4.6 ± 1.5	2.8 ± 1.1	2.3 ± 1.7

* Age at blood draw in years; $ levels in mmol/l.

Table 3. Summary of the heritability estimates of the four-variance component models for the 309 lipids and the 52 organic acids analyzed across all four metabolomics platforms.

The mean, median and range of the total heritability (h^2_{total}), heritability based on the 479 significant metabolite loci for the lipids or the 397 significant metabolite loci for the organic acids ($h^2_{Class\text{-}hits}$), the 596-683 significant metabolite loci not belonging to these classes ($h^2_{Notclass\text{-}hits}$) and the total heritability explained by metabolite loci (e.g., sum of $h^2_{Class\text{-}hits}$ and $h^2_{Notclass\text{-}hits}$: $h^2_{Metabolite\text{-}hits}$), as well as their standard errors (s.e.'s), are depicted for all 361 successfully analyzed metabolites as included on all platforms. **Supplementary Table 1** denotes which metabolites belong to each class and **Supplementary Table 3** provides the estimates for each of the individual metabolites.

		Lipids and lipid-like molecules		**Organic acids and derivatives**	
		estimate	**s.e.**	**estimate**	**s.e.**
$\mathbf{h^2_{total}}$	*mean*	0.47	0.04	0.41	0.04
	median	0.47	0.03	0.40	0.03
	range	(0.11 - 0.66)	(0.02 - 0.07)	(0.14 - 0.72)	(0.02 - 0.07)
$\mathbf{h^2_{Metabolite\text{-}hits}}$	*mean*	0.06	0.03	0.01	0.02
	median	0.06	0.03	0.02	0.02
	range	(-0.05 - 0.16)	(0.01 - 0.04)	(-0.08 - 0.11)	(0.01 - 0.04)
$\mathbf{h^2_{Class\text{-}hits}}$	*mean*	0.06	0.02	0.01	0.02
	median	0.06	0.02	0.01	0.02
	range	(-0.02 - 0.16)	(0.01 - 0.03)	(-0.04 - 0.14)	(0.01 - 0.03)
$\mathbf{h^2_{Notclass\text{-}hits}}$	*mean*	0.00	0.02	0.00	0.02
	median	0.01	0.02	0.00	0.02
	range	(-0.06 - 0.12)	(0.01 - 0.03)	(-0.06 - 0.05)	(0.01 - 0.03)

Table 4. Summary of the heritability estimates of the four-variance component models for the 17 essential and the 14 non-essential amino acids analyzed across all four metabolomics platforms.

The mean, median and range of the total heritability (h^2_{total}), and heritability based on the 397 significant metabolite loci for the organic acids ($h^2_{Class\text{-}hits}$), the 683 significant metabolite loci not belonging to this class ($h^2_{Notclass\text{-}hits}$) and the total heritability explained by metabolite loci (e.g., sum of $h^2_{Class\text{-}hits}$ and $h^2_{Notclass\text{-}hits}$: $h^2_{Metabolite\text{-}hits}$), as well as their standard errors (s.e.'s), are depicted for all 31 successfully analyzed essential and non-essential amino acids as included on all platforms. **Supplementary Table 1** denotes which metabolites belong to each class and **Supplementary Table 3** provides the estimates for each of the individual metabolites.

		Essential amino acids		**Non-essential amino acids**	
		estimate	**s.e.**	**estimate**	**s.e.**
	mean	0.42	0.04	0.39	0.04
$\mathbf{h^2_{total}}$	*median*	0.40	0.03	0.39	0.04
	range	(0.23 - 0.64)	(0.02 - 0.07)	(0.22 - 0.69)	(0.03 - 0.07)
	mean	0.00	0.02	0.02	0.03
$\mathbf{h^2_{Metabolite\text{-}hits}}$	*median*	0.00	0.02	0.01	0.03
	range	(-0.05 - 0.05)	(0.01 - 0.03)	(-0.07 - 0.11)	(0.01 - 0.04)
	mean	0.01	0.02	0.03	0.02
$\mathbf{h^2_{Class\text{-}hits}}$	*median*	0.00	0.02	0.01	0.02
	range	(-0.03 - 0.05)	(0.01 - 0.02)	(-0.03 - 0.14)	(0.01 - 0.03)
	mean	-0.01	0.02	0.00	0.02
$\mathbf{h^2_{Notclass\text{-}hits}}$	*median*	-0.01	0.02	0.00	0.02
	range	(-0.06 - 0.04)	(0.01 - 0.03)	(-0.04 - 0.03)	(0.01 - 0.03)