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. From the literature we aggregate > 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}), SNP-based heritability (h^2_{SNP}) and the proportion of heritability captured by known metabolite loci ($h^2_{GW\text{-}loci}$) for 309 lipids and 52 organic acids. Our study revealed significant differences in h^2_{SNP} and $h^2_{GW\text{-}loci}$ among different classes of lipids and organic acids. Furthermore, phosphatidylcholines with a higher degree of unsaturation had higher $h^2_{GW\text{-}loci}$ estimates. This study highlights the importance of common genetic variants for metabolite levels and elucidates the genetic architecture of metabolite classes and lipid species.

49 Metabolites are the small molecules involved in cellular metabolism, while the metabolome is typically
50 defined as the collection of metabolites produced by cells[1]. Metabolomics aims at providing a holistic
51 overview of the metabolome[1], and allows for the elucidation of underlying biological mechanisms and
52 metabolic disturbances in diseases. At the same time metabolomics may offer potential new therapeutic
53 targets or new biomarkers for disease diagnosis[2]. Variation in metabolite levels can arise due to gender[3],
54 and age[4], as well as physiologic effects, behavior, and lifestyle, such as diet[5]. Genetic differences may be
55 a source of direct variation in metabolomics profiles or may exert their effects on metabolite profiles
56 through the genetic influences on behavior or physiology.

57 Systematic investigations of common genetic variants in human metabolism by genome- and
58 metabolome-wide analysis successfully identified genetically influenced metabotypes (GIMs)[6]. The first
59 genome-wide association study (GWAS) in 2008 (*N* = 284 participants) identified four genetic variants
60 associated with metabolite levels[7]. Thereafter, GWAS with increasing sample sizes, and in diverse
61 populations, have resulted in the identification of hundreds of Single Nucleotide Polymorphism (SNP)
62 associations with metabolites from a wide range of metabolite classes[6]. Additional metabolite loci have
63 been identified by leveraging low-frequency and rare-variant analyses using (exome-) sequencing. We
64 conducted a comprehensive review of all quantitative trait locus (QTL) discovery for metabolites and
65 supply the complete reference list in **Supplementary Note 1**.

66 Twin and family studies estimated the heritability (h^2; proportion of phenotypic variance due to
67 genetic variance) for metabolite levels at around 50%, ranging from a heritability of 0% to 80% [5,8–15].
68 Several studies reported differences in heritability estimates among different classes of lipid species[12,14]
69 or lipoprotein subclasses[13]. For example, Rhee et al. (2013) reported higher heritability estimates for
70 amino acids than for lipids[11]. Essential amino acids, which cannot be synthesized by an organism *de*
71 *novo*[16], had lower heritability than non-essential amino acids[11] that are synthesized within the body[16].

72 Intriguingly, phosphatidylcholines[10] and triglycerides (TGs)[15] show increasing heritability as the number
73 of carbon atoms and/or double bonds in their fatty acyl side chains increases. Draisma et. al speculated
74 this might be attributed to differences in the number of metabolic conversion rounds for
75 phosphatidylcholines or TGs with a variable number of carbon atoms[10].

76 An improved understanding of the genetic architecture of intermediate phenotypes such as
77 metabolites may benefit insight into the aetiology of diseases and traits, such as cardiometabolic
78 diseases[17], migraine[18], psychiatric disorders[19], and cognition[20]. We aim to expand our understanding of
79 the contribution of genetic factors to variation in fasting blood metabolic measures (referred to as
80 metabolites in the remainder of the text for brevity) and analyzed data from multiple metabolomics
81 platforms from a large cohort of twins and family members (N = 5,117). Combining SNP and family data
82 allows for the simultaneous estimation of SNP heritability (h^2_{SNP}) and total heritability (h^2_{total})[21]. We
83 further extended this approach to estimate the proportion of variance explained by metabolite loci
84 identified by GWAS or rare-variant analysis ($h^2_{GW\text{-}loci}$; **Supplementary Data 1**). The $h^2_{GW\text{-}loci}$ consisted of
85 two sub-fractions, a fraction composed of all metabolite loci associated with metabolites of a specific
86 superclass ($h^2_{GW\text{-}Class}$) and a fraction composed of all other metabolite loci ($h^2_{GW\text{-}Notclass}$).

87 After characterizing all published metabolite-SNP associations by metabolite classification, we
88 present the h^2_{total}, h^2_{SNP} and $h^2_{GW\text{-}loci}$ results for 361 metabolites (**Figure 1**). Next, we further expand on
89 the current knowledge of the genetic aetiology of metabolite classes by employing mixed-effect meta-
90 regression models to test for differences in heritability estimates among metabolite classes and among
91 lipid species. To distinguish between the effects of the number of carbon atoms or number of double
92 bonds in the fatty acyl side chains of phosphatidylcholines and TGs additional univariate follow-up
93 analyses were conducted.

94 # Results

95 ## Metabolite classification

96 In the period of November 2008 to October 2018, 40 GWA and (exome-) sequencing studies have
97 identified 242,580 metabolite-SNP or metabolite ratio-SNP associations (see **Supplementary Note 1**).
98 These associations included 1,804 unique metabolites or ratios and 49,231 unique SNPs (43,830 after
99 converting all SNPs to build 37; **Supplementary Data 1**). For all metabolites their Human Metabolome
100 Database (HMDB)[22–24] identifiers were retrieved in order to extract information with regards to their
101 hydrophobicity and chemical classification (see **Methods**). Excluding the ratios and unidentified
102 metabolites, 953 metabolites could be classified into 12 'super classes' (**Table 1**), 43 'classes', or 77
103 'subclasses' based on the HMDB classification (**Supplementary Data 1**). The majority of the metabolites
104 were classified as 'lipids' and 'organic acids'. The 'lipids' could be subdivided into 8 classes, with 1 to
105 95,795 metabolite-SNP associations per class (mean = 17,589; SD = 32,553), and in 32 subclasses, with 1-
106 40,440 metabolites-SNP associations of per subclass (mean = 4,673; SD = 9,124). The 'organic acids and
107 derivatives' could be divided in 9 classes, with 1 to 26,832 metabolite-SNP associations per class (mean =
108 3,374; SD = 8,832), and 17 'organic acid' subclasses, including 1 to 26,448 metabolite-SNP associations
109 per subclass (mean = 1,786; SD = 6,371; **Supplementary Data 1**).

110 For 5,117 individuals, data were available from four different metabolomics platforms: the
111 Nightingale Health ^{1}H-NMR platform, a UPLC-MS Lipidomics platform, the Leiden ^{1}H-NMR platform and
112 the Biocrates Absolute-IDQ™ p150 platform. All participants were registered with the Netherlands Twin
113 Register (NTR)[25] and came from 2,445 nuclear families. Metabolomics and SNP data were available for
114 all participants. Background and demographic characteristics for the sample can be found in **Table 2**.
115 Across all four platforms 427 metabolites were assessed. After excluding the ratios (17) and the
116 metabolites of super classes not included in the curated metabolite-SNP association list (8), data were

117 available for 402 metabolites. The 402 metabolites could be classified as 336 'lipids', 53 'organic acids', 9
118 'organic oxygen compounds', 3 'proteins' and one 'organic nitrogen compound'. In the remainder of this
119 paper we solely focus on the 369 metabolites classified as 'lipids' or 'organic acids and derivatives'. The
120 full list of metabolites, with their classifications and the quartile values of the untransformed levels, are
121 included in **Supplementary Table 1**.

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

123 For the 369 metabolites that passed QC, we estimated total heritability (h^2_{total}), the proportion of
124 phenotypic variance explained by measured SNPs (h^2_{SNP}), the proportion attributable to metabolite
125 superclass-specific loci ($h^2_{GW\text{-}Class}$) and the proportion of variance attributable to non-superclass
126 metabolite loci ($h^2_{GW\text{-}Notclass}$) in twin and family members. The four-variance component analyses were
127 performed in the genome-wide complex trait analysis (GCTA) software[26]. The analyses were performed
128 separately for 'lipids' and 'organic acids', using unique superclass-specific and non-superclass genetic
129 relationship matrices (GRMs; created in LDAK[27,28]) in both sets of analyses (**Figure 1**). The 'lipid' analyses
130 employed a superclass-specific GRM of 479 'lipid' loci and a non-superclass GRM including 596 SNPs
131 (**Figure 1**). The 'organic acid' analyses included a superclass-specific GRM with 397 loci and a non-
132 superclass GRM with 683 SNPs (**Figure 1**). Before analyses, the metabolite data were normalized (log-
133 normal or inverse rank; **see Methods**). All models included age at blood draw, sex, the first 10 principal
134 components (PCs) from SNP genotype data, genotyping chip and metabolomics measurement batch as
135 covariates.

136 **Supplementary Table 2** includes the estimates for h^2_{total} , h^2_{SNP}, and $h^2_{GW\text{-}loci}$ from the four-
137 variance genetic component model for all 369 metabolites. The genomic relatedness matrix residual
138 maximum likelihood (GREML) algorithm converged successfully for 361 (97.8%) of the 53 'organic acids'
139 and 316 'lipids'. Poor convergence of the GREML algorithm was observed for 6 metabolites (1.6%). The

140 analyses for 2 metabolites (0.5%) were not completed due to non-invertible variance-covariance
141 matrices. The estimates for h^2_{total} of the 309 'lipids' ranged from 0.11 to 0.66 (mean = 0.47; mean s.e. =
142 0.04). The h^2_{SNP} estimates ranged from -0.54 to 0.71 (mean = 0.05; mean s.e. = 0.24). The estimates for
143 $h^2_{GW\text{-}loci}$ ranged from -0.05 to 0.16 (mean = 0.06; mean s.e. = 0.03; **Table 3**). The 52 'organic acids' had
144 h^2_{total} estimates ranging from 0.14 to 0.72 (mean = 0.41; mean s.e. = 0.04). The estimates for h^2_{SNP} ranged
145 from -0.42 to 0.46 (mean = 0.05; mean s.e. = 0.24) and for $h^2_{GW\text{-}loci}$ ranged from -0.08 to 0.11 (mean =
146 0.01; mean s.e. = 0.02; **Table 3**). On average, for both 'lipids' and 'organic acids' the h^2_{class} was higher
147 than the $h^2_{Notclass}$, with $h^2_{GW\text{-}Class}$ ranging from -0.02 to 0.16 (0.06; mean s.e. = 0.02) for 'lipids' and from -
148 0.04 to 0.14 for 'organic acids' (mean = 0.01; mean s.e. = 0.02). For both 'lipids' and 'organic acids' $h^2_{GW\text{-}}$
149 $_{Notclass}$ was zero (mean s.e. = 0.02), ranging from -0.06 to 0.12 for 'lipids' and from -0.06 to 0.05 for
150 'organic acids' (**Table 3**).

151 Including multiple metabolomics platforms allowed for a comparison of metabolites as
152 measured on multiple platforms. An earlier study showed 29 out of 43 overlapping metabolites across
153 two platforms to exhibit moderate heritability on both platforms[29]. In the current study, 61 metabolites
154 were measured on multiple platforms, with moderate h^2_{total} on each of the platforms and on average a
155 medium positive correlation between the h^2_{total} of the same metabolite assessed on different platforms
156 (mean r h^2_{total} = 0.36; **Supplementary Table 3**).

157 Differential heritability among metabolite classes and lipid-species

158 **Figure 2** shows variation in median heritability among the different classes of 'organic acids': 'keto
159 acids', 'hydroxy acids' and 'carboxylic acids' (see **Supplementary Table 1** for metabolites per class). 'Keto
160 acids', followed by 'carboxylic acids', had the highest median h^2_{total}, h^2_{SNP} and $h^2_{GW\text{-}Class}$ estimates (**Figure**
161 **2**). While 'hydroxy acids' had the highest median $h^2_{GW\text{-}Notclass}$ and $h^2_{GW\text{-}loci}$ estimates, the lowest median
162 h^2_{total}, h^2_{SNP} and $h^2_{GW\text{-}Class}$ estimates were observed for these metabolites (**Figure 2**). To investigate

163 whether heritability differs significantly among classes of 'organic acids', we applied multivariate mixed-
164 effect meta-regression, corrected for metabolite platform effects (see **Methods**). The multivariate
165 mixed-effect meta-regression models showed that h^2_{total} and $h^2_{GW\text{-}Class}$ for the 'organic acid' classes did
166 not differ significantly. Significant differences among the 'organic acid' classes, though, were observed
167 for the h^2_{SNP} estimates ($F(4, 47) = 7.48$, FDR-adjusted p-value = 0.02), the $h^2_{GW\text{-}loci}$ estimates ($F(4, 47) =$
168 3.44, FDR-adjusted p-value = 0.03), and the $h^2_{GW\text{-}Notclass}$ estimates ($F(4,47) = 19.95$, FDR-adjusted p-value
169 $= 1.25\text{x}10^{-08}$; **Supplementary Table 4**).

170 The multivariate mixed-effect meta-regressions were also applied to assess the significance of
171 heritability differences among essential and non-essential amino acids (subdivision of 'carboxylic acids';
172 see **Supplementary Table 5**) and among 'lipid' classes (see **Supplementary Table 1** for metabolites per
173 'lipid' class). None of the observed mean differences among essential and non-essential amino acids
174 (**Table 4**) were significant in the meta-regressions (**Supplementary Table 4**). Small but significant median
175 heritability differences were observed among the different classes of 'lipids' (**Figure 3**). For 'lipid' classes
176 the $h^2_{GW\text{-}loci}$ estimates differed significantly ($F(8, 300) = 8.47$; FDR-adjusted p-value = 0.004;
177 **Supplementary Table 4**).

178 Finally, we explored whether heritability of phosphatidylcholines and TGs increases with a larger
179 number of carbon atoms and/or double bonds in their fatty acyl side chains. To this end we employed
180 both uni- and multivariate mixed-effect meta-regression models separately for the TGs, diacyl
181 phosphatidylcholines (PCaa) and acyl-alkyl phosphatidylcholines (PCae; see **Methods**). The platform
182 specific heritability estimates for each of these lipid species has been depicted in **Supplementary Figure**
183 **1**. Variation in the number of carbon atoms and double bonds was significantly associated with $h^2_{GW\text{-}loci}$
184 estimates for PCaa's ($F(3, 52) = 7.05$; FDR-adjusted p-value = 0.009) and PCae's ($F(3, 45) = 3.41$; FDR-
185 adjusted p-value = 0.05; **Supplementary Table 4**). Phosphatidylcholines with a larger number of carbon

186 atoms showed lower heritability estimates and phosphatidylcholines with a larger number of double
187 bonds had higher heritability estimates (**Supplementary Table 4**). The differences among the
188 phosphatidylcholines with a variable number of carbon atoms and/or double bonds could be
189 contributed to differential h^2_{Class} estimates. Univariate models confirmed the pattern for the number of
190 double bonds in PCaa's and PCae, though they were not significant after correction for multiple testing
191 (**Supplementary Table 6**).

192 Discussion

193 We carried out a comprehensive assessment of GWA-metabolomics studies and created a repository of
194 all studies reporting on associations of SNPs and blood metabolites in European ancestry samples. This
195 led to 241,965 genome-wide associations that were curated, lifted to NCBI build 37 and for which all
196 associated metabolites were classified. The complete, categorized, overview of all blood metabolite-SNP
197 associations is provided in **Supplementary Data 1**, with the complete list of references in
198 **Supplementary Note 1**. The information from the repository served to construct six GRMs which then
199 served as predictors in the analysis of 369 metabolites. The metabolite data in our study derived from
200 four metabolomics platforms and two metabolite super classes. By mapping all metabolites to the
201 Human Metabolome Database (HMDB)[22–24] we were able to classify both the measured metabolites and
202 all previously published metabolites as either 'lipids' or 'organic acids'. Because the participants in the
203 study (N = 5,117) came from a large cohort of MZ and DZ twin-families we could evaluate the total
204 heritability (h^2_{total}) and the contributions of genome-wide SNPs (h^2_{SNP}) on 'lipids' and 'organic acids'. A
205 unique feature of the study was the ability to disentangle the role of superclass-specific ($h^2_{GW\text{-}Class}$) and
206 non-superclass ($h^2_{GW\text{-}Notclass}$) metabolite loci on heritability differences among metabolite classes and
207 lipid species.

208 To evaluate differences among metabolite classes and lipid species in the estimates for h^2_{total},
209 h^2_{SNP}, $h^2_{GW\text{-}loci}$, $h^2_{GW\text{-}Class}$, and $h^2_{GW\text{-}Notclass}$ multivariate mixed-effect meta-regression models were applied.
210 No significant differences in h^2_{total} estimates existed among any of the metabolite classes. Congruent
211 with a previous twin-family study[9], none of the heritability estimates differed significantly among
212 essential and non-essential amino acids. Both h^2_{SNP} and $h^2_{GW\text{-}loci}$ showed significant differences among
213 the different classes of 'organic acids'. 'Keto acids' had significantly higher h^2_{SNP} and significantly lower
214 $h^2_{GW\text{-}loci}$ estimates as compared with 'carboxylic acids'. Class-specific metabolite loci heritability
215 estimates for 'fatty acyls', 'lipoproteins' and 'steroids' were significantly higher. Similarly, significant
216 heterogeneity in lipid class heritability, with lower h^2_{total} and h^2_{SNP} for phospholipids than for
217 sphingolipids or glycerolipids has been described[12,14,30]. Lastly, we assessed whether heritability
218 increases with added complexity in lipid species[10,15]. We found that this indeed held for $h^2_{GW\text{-}loci}$
219 estimates in more complex diacyl and acyl-alkyl phosphatidylcholines but not for more complex TGs.
220 Previous research reported significant higher h^2_{SNP} estimates in polyunsaturated fatty acid containing
221 lipids[14]. Furthermore, loci of traditional lipid measures explained 2% to 21% of the variance in lipid
222 levels[14]. Together these results suggest that higher heritability in phosphatidylcholines is driven by a
223 lower number of carbon atoms and higher number of double bonds, e.g. a larger degree of
224 unsaturation.

225 Evaluating the mean heritability differences among 'lipids' and 'organic acids' it appears that
226 'lipids' have higher h^2_{total}, $h^2_{GW\text{-}Class}$ and $h^2_{GW\text{-}loci}$ estimates than 'organic acids' (**Table 3**). However, as the
227 GRMs used in the calculation of the heritability estimates differed among these classes, we were unable
228 to empirically compare mean differences. Comparison of our findings with those of previous twin-family
229 studies indicates that the heritability difference among 'lipids' and 'organic acid' is infrequently
230 investigated[8–11]. A possible explanation for the lack of comparisons may be the shortage of balanced
231 metabolomics platforms. The majority of metabolomics platforms have a strong focus on either 'lipids'

232 or ‘organic acids’, which complicates such comparisons. The disproportion of metabolite classes on
233 metabolomics platforms also affects the known metabolite loci, where ‘lipid’ studies have been
234 overrepresented as well. As a consequence, especially the $h^2_{GW\text{-}Class}$ and $h^2_{GW\text{-}loci}$ estimates of the ‘organic
235 acids’ will be underpowered due to this imbalance. For multi-component GREML our platform-specific
236 sample sizes were relatively small[31]. Only the Nightingale Health ^{1}H-NMR platform was sufficiently
237 powered to obtain small s.e.’s in single-component GREML using unrelated individuals with common
238 SNPs[32]. New[30,33–35] and future studies will increase the number of variants identified as metabolite loci.
239 The investment in UK Biobank[36] is expected to dramatically increase sample sizes for large-scale
240 genomic investigations of the human metabolome and subsequently the number of metabolite loci.

241 Applications such as two-sample Mendelian Randomization benefit greatly from the
242 comprehensive overview of metabolite loci we identified. The identified loci are interesting to explore as
243 instruments for metabolome-wide Mendelian Randomization studies of complex traits. Our work further
244 offers valuable insights into the role of common genetic variants in class specific differences among
245 metabolite classes and lipids species. Further research is required to elucidate the contribution of rare
246 genetic variants to metabolite levels and differences among metabolite classes. A reasonable approach
247 to tackle this issue could be to carry out a similar study in a large sample of whole-genome sequencing
248 (WGS) data. Such an approach, using MAF- and LD-stratified GREML analysis[31], identified additional
249 variance due to rare variants for height and BMI[37]. The extent to which our findings might generalize to
250 populations of non-European ancestry is uncertain, with replication among different ethnicities being
251 more likely for loci of common human metabolism pathways[38].

252 In conclusion, we contributed to the further elucidation of the genetic architecture of fasting
253 blood metabolite levels and to differences in the genetic architecture among metabolite classes.
254 Extending the GREML framework with the inclusion of known metabolite loci allowed us to

255 simultaneously estimate h^2_{total}, h^2_{SNP}, $h^2_{GW\text{-}Class}$ and $h^2_{GW\text{-}Notclass}$ for 361 metabolites. Significant differences
256 in h^2_{SNP} or $h^2_{GW\text{-}loci}$ estimates were observed among different classes of 'lipids' and 'organic acids' and for
257 more complex diacyl and acyl-alkyl phosphatidylcholines. Future studies need to also elucidate the
258 proportion of metabolite variation influenced by heritable and non-heritable lifestyle factors, which may
259 help delineate new personalized disease prevention or treatment strategies for complex disorders.

260 # Methods

261 ## Participants

262 At the Netherlands Twin Register (NTR)[39] metabolomics data for twins and family members as measured
263 in blood samples were available for 6,011 individuals of whom 5,667 were genotyped. The blood
264 samples for the four metabolomics experiments described in this study were mainly collected in
265 participants of the NTR biobank project[25,40]. Blood samples were collected after a minimum of two hours
266 of fasting (1.3%), with the majority of the samples collected after overnight fasting (98.7%). Fertile
267 women were bled in their pill-free week or on day 2-4 of their menstrual cycle. For the current paper,
268 we excluded participants if they were not of European ancestry, were on lipid-lowering medication at
269 the time of blood draw or if they had not adhered to the fasting protocol. The exact number of
270 exclusions per dataset is listed in **Supplementary Table 7**. After completing the preprocessing of the
271 metabolomics data, the separate subsets (e.g., different collection and measurement waves; see
272 **Supplementary Table 7**) of each platform were merged into a single per platform dataset, randomly
273 retaining a single observation per platform whenever multiple observations were available.
274 **Supplementary Table 8** gives an overview of the overlap in participants among the different platforms,
275 with the overlap among each metabolite that survived quality control (QC) for all four platforms
276 available in **Supplementary Table 9**. The final number of participants included in the study was 5,117,

277 with platform specific sample size ranging from 1,448 to 4,227 individuals from 946 to 2,179 families.
278 Characteristics for the individuals included in the analyses can be found in **Table 2**. Informed consent
279 was obtained from all participants. Projects were approved by the Central Ethics Committee on
280 Research Involving Human Subjects of the VU University Medical Centre, Amsterdam, an Institutional
281 Review Board certified by the U.S. Office of Human Research Protections (IRB number IRB00002991
282 under Federal-wide Assurance- FWA00017598; IRB/institute codes, NTR 03-180 and EMIF-AD 2014.210).

283 Metabolite profiling

284 Nightingale Health ^{1}H-NMR platform

285 Metabolic biomarkers were quantified from plasma samples using high-throughput proton nuclear
286 magnetic resonance spectroscopy (^{1}H-NMR) metabolomics (Nightingale Health Ltd, Helsinki, Finland;
287 formerly Brainshake Ltd.). This method provides simultaneous quantification of routine lipids,
288 lipoprotein subclass profiling with lipid concentrations within 14 subclasses, fatty acid composition, and
289 various low-molecular weight metabolites including amino acids, ketone bodies and glycolysis-related
290 metabolites in molar concentration units. Details of the experimentation and epidemiological
291 applications of the NMR metabolomics platform have been reviewed previously[41,42].

292 UPLC-MS lipidomics platform

293 Plasma lipid profiling was performed at the division of Analytical Biosciences at the Leiden Academic
294 Center for Drug Research at Leiden University/Netherlands Metabolomics Centre. The lipids were
295 analyzed with an Ultra-High Performance Liquid Chromatograph directly coupled to an Electrospray
296 Ionization Quadruple Time-of-Flight high resolution mass spectrometer (UPLC-ESI-Q-TOF; Agilent 6530,
297 San Jose, CA, USA) that uses reference mass correction. For liquid chromatographic separation a
298 ACQUITY UPLC HSS T3 column (1.8µm, 2.1 ∗ 100mm) was used with a flow of 0.4 ml/min over a 16
299 minute gradient. Lipid detection was done using a full scan in the positive ion mode. The raw MS data

300 were pre-processed using Agilent MassHunter Quantitative Analysis software (Agilent, Version B.04.00).
301 Detailed descriptions of lipid profiling and quantification have been described previously[43,44].

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

303 The Leiden ^{1}H-NMR spectroscopy experiment of EDTA-plasma samples used a 600 MHz Bruker Advance
304 II spectrometer (Bruker BioSpin, Karlsruhe, Germany). The peak deconvolution method used for this
305 platform has been previously described[45].

306 Biocrates Absolute-IDQ™ p150 platform

307 The Biocrates Absolute-IDQ™ p150 (Biocrates Life Sciences AG, Innsbruck, Austria) metabolomics
308 platform on serum samples was analysed at the Metabolomics Facility of the Genome Analysis Centre at
309 the Helmholtz Centre in Munich, Germany. This platform utilizes flow injection analysis coupled to
310 tandem mass spectrometry (MS/MS) and has been described in detail elsewhere[3,46,47].

311 Metabolomics data preprocessing

312 Preprocessing of the metabolomics data was done for each of the platforms and measurement batches
313 per platform separately. Metabolites were excluded from analysis when the mean coefficient of
314 variation exceeded 25% and the missing rate exceeded 5%. Metabolite measurements were set to
315 missing if they were below the lower limit of detection or quantification or could be classified as an
316 outlier (five standard deviations greater or smaller than the mean). Metabolite measurements that were
317 set to missing because they fell below the limit of detection/quantification were imputed with half of
318 the value of this limit, or when this limit was unknown with half of the lowest observed level for this
319 metabolite. All remaining missing values were imputed using multivariate imputation by chained
320 equations ('mice')[48]. On average, 9 values had to be imputed for each metabolites (SD = 12; range: 1-
321 151). Data for each metabolite on both ^{1}H-NMR platforms were normalized by inverse normal rank
322 transformation[45,49], while the imputed values of the Biocrates metabolomics platform and the UPLC-MS

323 lipidomics platform were normalized by natural logarithm transformation[10,50], conform previous
324 normalization strategies applied to the data obtained using these platforms. The complete lists with full
325 names of all detected metabolites that survived QC and preprocessing for all platforms can be found in
326 **Supplementary Table 1**, these tables also include the quartile values of the untransformed metabolites.

327 Genotyping, imputation and ancestry outlier detection

328 Genotype information was available for 21,001 NTR participants for 6 different genotyping arrays (Affymetrix
329 6.0 [*N* = 8,640], Perlegen-Affymetrix [*N* = 1,238], Illumina Human Quad Bead 660 [*N* = 1,439], Affymetrix
330 Axiom [*N* = 3,144], Illumnia GSA [*N* = 5,938] and Illumina Omni Express 1M [*N* =238]), as well as sequence
331 data from the Netherlands reference genome project GONL (BGI full sequence at 12x (*N* = 364)[51]. For each
332 genotyping array samples were removed if they had a genotype call rate above 90%, gender-mismatch
333 occurred or if heterozygosity (Plink F statistic) fell outside the range of -0.10 – 0.10. SNPs removed if they
334 were palindromic AT/GC SNPs with a minor allele frequency (MAF) range between 0.4 and 0.5, when the MAF
335 was below 0.01, when Hardy Weinberg Equilibrium (HWE) had $p < 10^{-5}$, when the number of Mendelian
336 errors was greater than 20 and the genotype call rate was < 0.95. After QC the six genotyping arrays were
337 aligned to the GONL reference set (V4) and SNPs were removed if the alleles mismatched with this reference
338 panel or the allele frequency different more than 0.10 between the genotyping array and this reference set.
339 The data from the six genotyping chips were subsequently merged into a single dataset (1,781,526
340 SNPs). Identity-by-decent (IBD) was estimated with PLINK[52] and KING[53] for all individual pairs based on the
341 ~10.6K SNPs in common across the arrays, next IBD was compared to expected family relations and
342 individuals were removed if this mismatched. Prior to imputation to the GONL reference data[54,55] the
343 duplicate monozygotic pairs (*N* = 3,032) or trios (*N* = 7) and NTR GONL samples (*N* = 364) were removed and
344 the data was cross-array phased using MACH-ADMIX[56]. Post-imputation the NTR GONL samples and the
345 duplicated MZ pairs and trios were re-added to the data. Filtering of the imputed dataset included the
346 removal of SNPs that were significantly associated with a single genotyping chip ($p < 10^{-5}$), had HWE $p < 10^{-5}$,

347 the Mendelian error rate > mean + 3 SD or if the imputation quality (R^2) was below 0.90. The final cross-
348 platform imputed dataset included 1,314,639 SNPs, including 20,792 SNPs on the X-chromosome.
349 The cross-platform imputed data was aligned with PERL based "HRC or 1000G Imputation preparation
350 and checking" tool (version 4.2.5; https://www.well.ox.ac.uk/~wrayner/tools). The remaining 1,302481 SNPs
351 were phased with EAGLE[57] for the autosomes, and SHAPEIT[58] for chromosome X and then imputed to 1000
352 Genomes Phase 3 (1000GP3 version 5)[59] on the Michigan Imputation server using Minimac3 following the
353 standard imputation procedures of the server[60]. Principal Component Analysis (PCA) was used to project the
354 first 10 PCs of the 1000 genomes references set population on the NTR cross-platform imputed data using
355 SMARTPCA[61]. Ancestry outliers (non-Dutch ancestry; N = 1,823) were defined as individuals with PC values
356 outside the European/British population range[62]. After ancestry outlier removal the first 10 PCs were
357 recalculated.

358 Curation of metabolite loci

359 In October 2018 PubMed and Google Scholar were searched to identify published GWA and (exome-)
360 sequencing studies on metabolomics or fatty acid metabolism in blood samples using ^{1}H-NMR, mass
361 spectrometry or gas chromatography-based methods. In the period of November 2008 to October 2018
362 40 GWA or (exome-) sequencing studies on blood metabolomics in European samples have been
363 published (**Supplementary Note 1**). For all studies the genome-wide significant ($p < 5x10^{-8}$) metabolite-
364 SNP associations were extracted, including only those observations for autosomal SNPs and reporting
365 SNP effect sizes and p-values based on the summary statistics excluding NTR samples were relevant[49,50].
366 Across the 40 studies, 242,580 metabolite-SNP or metabolite ratio-SNP associations were reported,
367 these associations included 1,804 unique metabolites or ratios and 49,231 unique SNPs (**Supplementary**
368 **Data 1**). For all metabolites their Human Metabolome Database (HMDB)[22–24], PubChem[63], Chemical
369 Entities of Biological Interest (ChEBI)[64] and International Chemical Identifier (InChiKey)[65] identifiers have
370 been retrieved. Information with regards to the 'super class', 'class' and 'subclass' of metabolites was

371 extracted from HMDB, whenever no HMDB identifier was available and categorization information could
372 not be extracted, 'super class', 'class' and 'subclass' were provided based on expert opinion. Excluding
373 the ratios and unidentified metabolites, 953 metabolites could be classified into 12 'super classes', 43
374 'classes' or 77 'subclasses' (**Supplementary Data 1**). Based on the metabolite identifiers we also
375 extracted the *log(S)* value for each metabolite to assess the hydrophobicity of the metabolites. The
376 *log(S)* value represents the log of the partition coefficient between 1-octanol and water, two fluids that
377 hardly mix. The partition coefficient is the ratio of concentrations in water and in octanol when a
378 substance is added to an octanol-water mixture and hence indicates the hydrophobicity of a compound.
379 Thus, we classify a metabolite as hydrophobic if it is more hydrophobic than 1-octanol itself and
380 hydrophilic otherwise (**Supplementary Data 1**).

381 The 49,231 unique SNPs reported their rsIDs or chromosome-base pair positions by different
382 genome builds or dbSNP maps[66], therefore we lifted all SNPs to HG19 build 37[67], after which 43,830
383 unique SNPs remained (**Figure 1**; **Supplementary Data 1**). All bi-allelic metabolite SNPs were extracted
384 from our 1000GP3 data, which excluded 295 tri-allelic SNPs and 4,256 SNPs could not be retrieved from
385 1000GP3. Next, MAF > 1% (2,067 SNPs removed), $R^2 > 0.70$ (2,002 SNPs) and HWE $P < 10^{-4}$ (72 SNPs)
386 filtering was performed, resulting in 35,138 metabolite SNPs for NTR participants (**Figure 1**). Next, we
387 created two 'super class'-specific lists of metabolite loci and two 'not-superclass' lists of metabolite loci.
388 To create a list of loci for the 652 unique metabolites classified as 'lipids and lipid-like molecules' (e.g.,
389 'lipids'), in 2,500 unrelated individuals we clumped (PLINK version 1.9) all 112,760 lipid-SNP associations
390 using an LD-threshold (r^2) of 0.10 in a 500kb radius (**Figure 1**). Clumping identified 482 lead SNPs, or loci,
391 for 'lipids' and an additional 12,169 SNPs were identified as LD-proxies for the lipid-loci (**Figure 1**). To
392 obtain the 'not-superclass' list of lipid loci the 12,651 lipid loci and proxies were removed from the list of
393 all metabolite-SNP associations and the resulting list was clumped to obtain the 598 'non-superclass' loci

394 (**Figure 1**). The same clumping procedure was applied to the 26,352 organic acid-SNP associations,
395 identifying 398 organic acids loci, 10,781 organic acid LD-proxies and 687 'non-superclass' loci (**Figure 1**).

396 Construction of genetic relationship matrices

397 In total six weighted genetic relationship matrixes (GRMs) were constructed, which were corrected for
398 uneven and long-range LD between the SNPs (LDAK version 4.9[27,28]; **Figure 1**). In **Supplementary Note2** the
399 use of weighted versus unweighted GRMs is compared using simulations. Two of the GRMs used the cross-
400 platform imputed dataset as backbone and the other four GRMs were based on SNPs extracted from the
401 1000GP3 imputed data. For inclusion in the first GRM, after removal of ancestry outliers, the autosomal SNPs
402 of the cross-platform imputed dataset were filtered on MAF (<1%) and all lipid and organic acid loci, their LD-
403 proxies and 50kb surrounding both types of SNPs were removed (see **curation of metabolite loci**; **Figure 1**).
404 The resulting LDAK GRM included 434,216 SNPs and the *V(G1)* variance component in the genomic
405 relatedness matrix residual maximum likelihood (GREML) analyses is based on this GRM (see **heritability**
406 **analyses**; **Figure 1**). The *V(G2)* variance component in the GREML analyses is based on the LDAK GRM
407 including all autosomal SNPs with a MAF greater than 1% included on the cross-platform imputed dataset
408 (447,794 SNPs), where ancestry outliers were removed and for all individual pairs sharing less than 0.05 of
409 their genome their sharing was set to zero[21] (**Figure 1**). Depending on the metabolite the *V(G3)* variance
410 component in the GREML analyses was either based on an LDAK GRM of the 1000GP3 extracted lipid loci (479
411 SNPs) or the organic acid loci (397 SNPs; **Figure 1**). Finally, depending on the metabolite either the 'not-lipid'
412 LDAK GRM (596 SNPs) or the 'not-organic acid' LDAK GRM (683 SNPs) underlay the *V(G4)* variance component
413 in the GREML analyses (**Figure 1**). **Supplementary Data 1** indicates for each listed SNP if it was included in any
414 of the LDAK GRMs.

415 ## Statistical analyses

416 ### Heritability analyses

417 Mixed linear models[21], implemented in the genome-wide complex trait analysis (GCTA) software
418 package (version 1.91.7)[26], were applied to compare three models including a variable number of
419 covariates. **Supplementary Table 10** gives the three different models, full descriptions of the covariates
420 and model comparison have been given in **Supplementary Note 3**. The mean and median h^2_{total} and h^2_{SNP}
421 estimates and standard errors were highly similar across the different models, as such the most sparse
422 model was chosen for further analyses (**Supplementary Table 11**). This final model included the first 10
423 genetic PCs for the Dutch population, genotyping chip, sex and age at blood draw as covariates. For
424 metabolites of the Nightingale Health ^{1}H-NMR and Biocrates platform, measurement batch was included
425 as covariate.

426 The final four-variance component model including four GRMs, allowing the estimation of the
427 proportion of variation explained by superclass-specific significant metabolite loci ($h^2_{GW\text{-}Class}$) and non-
428 superclass significant metabolite loci ($h^2_{GW\text{-}Notclass}$) in addition to estimating the h^2_{SNP} and total h^2 (h^2_{total};
429 **Figure 1**). In this extension, the total variance explained by significant metabolite loci ($h^2_{GW\text{-}loci}$) consists
430 of the sum of $\frac{V(G3)}{Vp}$ and $\frac{V(G4)}{Vp}$, where *Vp* is the phenotypic variance and h^2_{SNP} is defined as the sum of
431 $\frac{V(G1)}{Vp}$, $\frac{V(G3)}{Vp}$ and $\frac{V(G4)}{Vp}$ (**Figure 1**). To calculate the standard errors (s.e.'s) for the composite variance
432 estimates, we have randomly sampled 10,000 instances from the parameter variance-covariance
433 matrices for each metabolite. The s.e.'s of the specific ratio of interest were then based on the standard
434 deviation of the ratio of interest across 10.000 samples. The four-variance component models obtained
435 the unconstrained variance components which allowed for negative h^2_{SNP} and $h^2_{GW\text{-}loci}$ estimates. All four-
436 variance component models applied the --reml-bendV flag where necessary to invert the variance-
437 covariance matrix *V* if *V* was not positive definite, which may occur when variance components are

438 negative[68]. Finally, we calculated the log likelihood of a reduced model with either *V(G3)*, *V(G4)* or both
439 dropped from the full model and calculated the LRT and p-value (**Supplementary Table 2**).

440 ### Mixed-effect meta-regression analyses

441 To investigate differences in heritability estimates among metabolites of different classes we applied
442 mixed-effect meta-regression models as implemented in the 'metafor' package (version 2.0-0) in R
443 (version 3.5.1)[69]. Here we tested for the moderation of heritability estimates by metabolite class and
444 metabolomics platform on all 361 successfully analyzed metabolites while including a matrix combining
445 the phenotypic correlations (**Supplementary Table 12**) and the sample overlap (**Supplementary Table 9**)
446 between the metabolites as random factor to correct for dependence among the metabolites and
447 participants. This matrix includes the sample size of the metabolite on the diagonal, with the off-
448 diagonal computed by $\frac{N_{1,2}}{\sqrt{n_1 * n_2}} * r$ (**Supplementary Table 13**), where $N_{1,2}$ is the sample overlap between
449 the metabolites, n_1 is the sample size of metabolite one, n_2 is the sample size of metabolite two and *r* is
450 the phenotypic correlation between the metabolites as calculated with Spearman's Rho. For all mixed-
451 effect meta-regression models we obtained the robust estimates based on a sandwich-type estimator,
452 clustered by the metabolites included in the models to correct for the sample overlap among the
453 different metabolites[70]. First, we used multivariate mixed-effect meta-regression models to
454 simultaneously estimate the effect of metabolite class and metabolomics platform on the h^2_{total}, h^2_{SNP}
455 and the $h^2_{GW\text{-}loci}$, as well as the $h^2_{GW\text{-}Class}$ and $h^2_{GW\text{-}Notclass}$ estimates. Subsequently, to separately assess the
456 effect of the number of carbon atoms or double bonds in the fatty acyls chains of phosphatidylcholines
457 and triglycerides univariate models were conducted as follow-up. To account for multiple testing the p-
458 values were adjusted with the with the False Discovery Rate (FDR)[71] using the 'p.adjust' function in R.
459 Multiple testing correction was done separately for the univariate and the multivariate models.

460 ## Data availability

461 The curated list of all published metabolite-SNP associations is included in **Supplementary Data 1** and is
462 publicly available through the BBMRI – omics atlas (http://bbmri.researchlumc.nl/atlas/#data). All
463 information on the metabolites in this study are in **Supplementary Table 1**; with full summary statistics
464 for the four-variance component models included in **Supplementary Table 2**. The Nightingale Health
465 metabolomics data may be requested through BBMRI-NL (https://www.bbmri.nl/Omics-metabolomics).
466 All (other) data may be accessed, upon approval of the data access committee, through the Netherlands
467 Twin Register (ntr.fgb@vu.nl). A reporting summary for this Article is available as Supplementary
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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.

Competing interests statement

The authors declare no competing financial interests.

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References

1. Patti, G. J., Yanes, O. & Siuzdak, G. Innovation: Metabolomics: the apogee of the omics trilogy. *Nat. Rev. Mol. Cell Biol.* **13**, 263–269 (2012).

2. Kuehnbaum, N. L. & Britz-McKibbin, P. New advances in separation science for metabolomics: resolving chemical diversity in a post-genomic era. *Chem. Rev.* **113**, 2437–68 (2013).

3. Mittelstrass, K. *et al.* Discovery of sexual dimorphisms in metabolic and genetic biomarkers. *PLoS Genet.* **7**, e1002215 (2011).

4. Chaleckis, R., Murakami, I., Takada, J., Kondoh, H. & Yanagida, M. Individual variability in human blood metabolites identifies age-related differences. *Proc. Natl. Acad. Sci. U. S. A.* **113**, 4252–4259 (2016).

5. Menni, C. *et al.* Targeted metabolomics profiles are strongly correlated with nutritional patterns in women. *Metabolomics* **9**, 506–514 (2013).

6. Kastenmüller, G., Raffler, J., Gieger, C. & Suhre, K. Genetics of human metabolism: an update. *Hum. Mol. Genet.* **24**, R93–R101 (2015).

7. Gieger, C. *et al.* Genetics meets metabolomics: a genome-wide association study of metabolite profiles in human serum. *PLoS Genet.* **4**, e1000282 (2008).

8. Nicholson, G. *et al.* Human metabolic profiles are stably controlled by genetic and environmental variation. *Mol. Syst. Biol.* **7**, 525 (2011).

9. Shah, S. H. *et al.* High heritability of metabolomic profiles in families burdened with premature cardiovascular disease. *Mol. Syst. Biol.* **5**, 258 (2009).

10. Draisma, H. H. M. *et al.* Familial resemblance for serum metabolite concentrations. *Twin Res. Hum. Genet.* **16**, 948–61 (2013).

11. Rhee, E. P. *et al.* A genome-wide association study of the human metabolome in a community-based cohort. *Cell Metab.* **18**, 130–143 (2013).

12. Frahnow, T. *et al.* Heritability and responses to high fat diet of plasma lipidomics in a twin study. *Sci. Rep.* **7**, 1–11 (2017).

13. Kaess, B. *et al.* The lipoprotein subfraction profile: heritability and identification of quantitative trait loci. *J. Lipid Res.* **49**, 715–723 (2008).

14. Bellis, C. *et al.* Human Plasma Lipidome Is Pleiotropically Associated With Cardiovascular Risk Factors and Death. *Circ. Cardiovasc. Genet.* **7**, 854–863 (2014).

15. Draisma, H. H. M. Analysis of Metabolomics Data from Twin Families. (Leiden, 2011).

16. Reeds, P. J. Dispensable and Indispensable Amino Acids for Humans. *J. Nutr.* **130**, 1874S–1876S (2000).

17. Newgard, C. B. Metabolomics and Metabolic Diseases: Where Do We Stand? *Cell Metab.* **25**, 43–56 (2017).

18. Onderwater, G. L. J. *et al.* Large-scale plasma metabolome analysis reveals alterations in HDL metabolism in migraine. *Neurology* **0**, 10.1212/WNL.0000000000007313 (2019).

19. Nedic Erjavec, G. *et al.* Short overview on metabolomic approach and redox changes in psychiatric disorders. *Redox Biol.* **14**, 178–186 (2018).

20. van der Lee, S. J. *et al.* Circulating metabolites and general cognitive ability and dementia:

Evidence from 11 cohort studies. *Alzheimer's Dement.* 1–16 (2018). doi:10.1016/j.jalz.2017.11.012

21. Zaitlen, N. *et al.* Using Extended Genealogy to Estimate Components of Heritability for 23 Quantitative and Dichotomous Traits. *PLoS Genet.* **9**, (2013).

22. Wishart, D. S. *et al.* HMDB: a knowledgebase for the human metabolome. *Nucleic Acids Res.* **37**, D603-10 (2009).

23. Wishart, D. S. *et al.* HMDB 3.0-The Human Metabolome Database in 2013. *Nucleic Acids Res.* **41**, 801–807 (2013).

24. Wishart, D. S. *et al.* HMDB 4.0: The human metabolome database for 2018. *Nucleic Acids Res.* **46**, D608–D617 (2018).

25. Willemsen, G. *et al.* The Netherlands Twin Register biobank: a resource for genetic epidemiological studies. *Twin Res. Hum. Genet.* **13**, 231–45 (2010).

26. Yang, J., Lee, S. H., Goddard, M. E. & Visscher, P. M. GCTA: A tool for genome-wide complex trait analysis. *Am. J. Hum. Genet.* **88**, 76–82 (2011).

27. Speed, D., Hemani, G., Johnson, M. R. & Balding, D. J. Improved heritability estimation from genome-wide SNPs. *Am. J. Hum. Genet.* **91**, 1011–1021 (2012).

28. Speed, D., Cai, N., Johnson, M. R., Nejentsev, S. & Balding, D. J. Reevaluation of SNP heritability in complex human traits. *Nat. Genet.* (2017). doi:10.1038/ng.3865

29. Yet, I. *et al.* Genetic influences on metabolite levels: A comparison across metabolomic platforms. *PLoS One* **11**, (2016).

30. Tabassum, R. *et al.* Genetics of human plasma lipidome: Understanding lipid metabolism and its link to diseases beyond traditional lipids. *bioRxiv* (2018). doi:10.1101/457960

31. Yang, J. *et al.* Genetic variance estimation with imputed variants finds negligible missing heritability for human height and body mass index. *Nat. Genet.* **47**, 1114–1120 (2015).

32. Visscher, P. M. *et al.* Statistical Power to Detect Genetic (Co)Variance of Complex Traits Using SNP Data in Unrelated Samples. *PLoS Genet.* **10**, (2014).

33. Gallois, A. *et al.* A comprehensive study of metabolite genetics reveals strong pleiotropy and heterogeneity across time and context. *bioRxiv* (2018). doi:http://dx.doi.org/10.1101/461848

34. Wittemans, L. B. L. *et al.* Assessing the causal association of glycine with risk of cardio-metabolic diseases. *Nat. Commun.* **10**, 1–13 (2019).

35. Demirkan, A. *et al.* Genome-wide association study of plasma lipids. *bioRxiv* (2019). doi:http://dx.doi.org/10.1101/621334

36. Sudlow, C. *et al.* UK Biobank: An Open Access Resource for Identifying the Causes of a Wide Range of Complex Diseases of Middle and Old Age. *PLoS Med.* **12**, 1–10 (2015).

37. Wainschtein, P. *et al.* Recovery of trait heritability from whole genome sequence data. *bioRxiv* (2019). doi:http://dx.doi.org/10.1101/588020

38. Yousri, N. A. *et al.* Whole-exome sequencing identifies common and rare variant metabolic QTLs in a Middle Eastern population. *Nat. Commun.* **9**, 1–13 (2018).

39. Boomsma, D. I. *et al.* Netherlands Twin Register: from twins to twin families. *Twin Res. Hum. Genet.* **9**, 849–57 (2006).

40. Willemsen, G. *et al.* The Adult Netherlands Twin Register: twenty-five years of survey and biological data collection. *Twin Res. Hum. Genet.* **16**, 271–81 (2013).

41. Soininen, P., Kangas, A. J., Würtz, P., Suna, T. & Ala-Korpela, M. Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Cardiovascular Epidemiology and Genetics. *Circ. Cardiovasc. Genet.* **8**, 192–206 (2015).

42. Würtz, P. *et al.* Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Large-Scale Epidemiology: A Primer on -Omic Technology. *Am. J. Epidemiol.* **186**, 1–13 (2017).

43. Gonzalez-Covarrubias, V. *et al.* Lipidomics of familial longevity. *Aging Cell* **12**, 426–434 (2013).

44. Dane, A. D. *et al.* Integrating metabolomics profiling measurements across multiple biobanks. *Anal. Chem.* **86**, 4110–4114 (2014).

45. Demirkan, A. *et al.* Insight in Genome-Wide Association of Metabolite Quantitative Traits by Exome Sequence Analyses. *PLoS Genet.* **11**, e1004835 (2015).

46. Goek, O. N. *et al.* Serum metabolite concentrations and decreased GFR in the general population. *Am. J. Kidney Dis.* **60**, 197–206 (2012).

47. Römisch-Margl, W. *et al.* Procedure for tissue sample preparation and metabolite extraction for high-throughput targeted metabolomics. *Metabolomics* **8**, 133–142 (2012).

48. Buuren, S. van & Groothuis-Oudshoorn, K. mice: Multivariate Imputation by Chained Equations in R. *J. Stat. Softw.* **45**, (2011).

49. Kettunen, J. *et al.* Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. *Nat. Commun.* **7**, 11122 (2016).

50. Draisma, H. H. M. *et al.* Genome-wide association study identifies novel genetic variants contributing to variation in blood metabolite levels. *Nat. Commun.* **6**, 7208 (2015).

51. Boomsma, D. I. *et al.* The Genome of the Netherlands: design, and project goals. *Eur. J. Hum. Genet.* **22**, 221–227 (2014).

52. Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).

53. Manichaikul, A. *et al.* Robust relationship inference in genome-wide association studies. *Bioinformatics* **26**, 2867–2873 (2010).

54. Fedko, I. O. *et al.* Estimation of Genetic Relationships Between Individuals Across Cohorts and Platforms: Application to Childhood Height. *Behav. Genet.* **45**, 514–528 (2015).

55. Deelen, P. *et al.* Improved imputation quality of low-frequency and rare variants in European samples using the 'Genome of the Netherlands'. *Eur. J. Hum. Genet.* **22**, 1321–1326 (2014).

56. Liu, E. Y., Li, M., Wang, W. & Li, Y. MaCH-Admix: Genotype Imputation for Admixed Populations. *Genet. Epidemiol.* **37**, 25–37 (2013).

57. Loh, P. R., Palamara, P. F. & Price, A. L. Fast and accurate long-range phasing in a UK Biobank cohort. *Nat. Genet.* **48**, 811–816 (2016).

58. Delaneau, O., Marchini, J. & Zagury, J.-F. A linear complexity phasing method for thousands of genomes. *Nat. Methods* **9**, 179–81 (2012).

59. Auton, A. *et al.* A global reference for human genetic variation. *Nature* **526**, 68–74 (2015).

60. Das, S. *et al.* Next-generation genotype imputation service and methods. *Nat. Genet.* **48**, 1284–

1287 (2016).

61. Price, A. L. *et al.* Principal components analysis corrects for stratification in genome-wide association studies. *Nat. Genet.* **38**, 904–909 (2006).

62. Abdellaoui, A. *et al.* Population structure, migration, and diversifying selection in the Netherlands. *Eur. J. Hum. Genet.* **21**, 1277–1285 (2013).

63. Kim, S. *et al.* PubChem 2019 update: Improved access to chemical data. *Nucleic Acids Res.* **47**, D1102–D1109 (2019).

64. Hastings, J. *et al.* ChEBI in 2016: Improved services and an expanding collection of metabolites. *Nucleic Acids Res.* **44**, D1214–D1219 (2016).

65. Heller, S. R., McNaught, A., Pletnev, I., Stein, S. & Tchekhovskoi, D. InChI, the IUPAC International Chemical Identifier. *J. Cheminform.* **7**, 1–34 (2015).

66. Sherry, S. T. *et al.* dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res.* **29**, 308–11 (2001).

67. Haeussler, M. *et al.* The UCSC Genome Browser database: 2019 update. *Nucleic Acids Res.* **47**, D853–D858 (2019).

68. Hayes, J. F. & Hill, W. G. Modification of Estimates of Parameters in the Construction of Genetic Selection Indices (' Bending '). *Biometrics* **37**, 483–493 (1981).

69. Viechtbauer, W. Conducting Meta-Analyses in R with the metafor Package. *J. Stat. Softw.* **36**, 1–48 (2010).

70. Hedges, L. V., Tipton, E. & Johnson, M. C. Robust variance estimation in meta-regression with

dependent effect size estimates. *Res. Synth. Methods* **1**, 39–65 (2010).

71. Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society B* **57**, 289–300 (1995).

Figures

Figure 1. Flowchart describing the filtering of metabolite SNPs, GRM construction and 4-variance component models.

This flowchart describes how the 242,580 metabolite-SNP associations as identified from GWA and rare-variant analyses (**Supplementary Note 1**; **Supplementary Data 1**) were converted to NCBI build 37, extracted for NTR participants from the 1000GP3 imputed data and filtered on MAF, HWE and R^2 (blue boxes at top of the figure indicated by the red curly bracket). The metabolite-SNP associations of the filtered SNPs were clumped ($r^2 = 0.10$) to obtain the metabolite loci and LD-proxies of the lipid and the organic acids, respectively (blue). To obtain the non-superclass loci, the superclass-specific loci and LD-proxies were removed from the overall list of metabolite-SNP associations and prior to clumping (blue). The lipid-loci, not-lipid loci, organic acid loci and not-organic acid loci give rise to four GRMs, respectively, as indicated by the black boxes and arrows in the flowchart. The two additional GRMs included in the 4-variance component GREML models are based on the cross-platform imputed SNPs (see **Methods**), where the lipid and organic acid loci, LD-proxies and 50 kb surrounding these SNPs have been removed from one of the cross-platform GRMs (black boxes in flowchart). The bottom part (in orange) of the flowchart describes the 4-variance component GREML model separately for the lipid and organic acid analyses (indicated by red curly brackets). To indicate which GRMs are used to calculate which variance components orange arrows have been drawn from the GRMs to the variance components. The different (combinations) of variance components give rise to the five different heritability estimates (h^2_{total}, h^2_{SNP}, $h^2_{GW\text{-}Class}$, $h^2_{GW\text{-}Notclass}$ and $h^2_{GW\text{-}loci}$), the final part of the flowcharts provides an overview of how these heritability estimates are derived (orange).

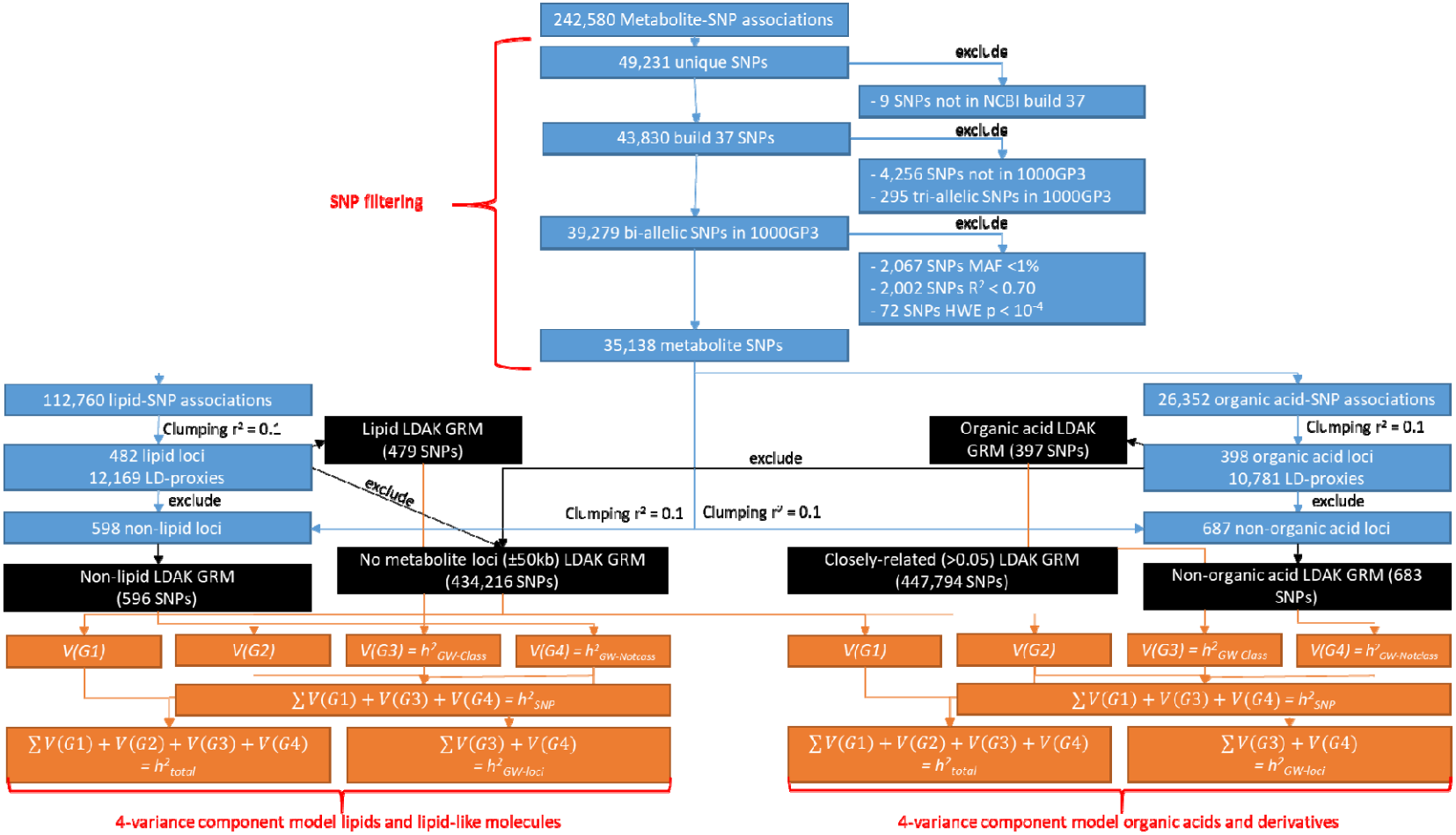

242,580 Metabolite-SNP associations
49,231 unique SNPs
exclude
- 9 SNPs not in NCBI build 37
43,830 build 37 SNPs
exclude
- 4,256 SNPs not in 1000GP3
- 295 tri-allelic SNPs in 1000GP3
SNP filtering
39,279 bi-allelic SNPs in 1000GP3
exclude
- 2,067 SNPs MAF <1%
- 2,002 SNPs R² < 0.70
- 72 SNPs HWE p < 10⁻⁴
35,138 metabolite SNPs
112,760 lipid-SNP associations
Clumping r² = 0.1
482 lipid loci
12,169 LD-proxies
exclude
598 non-lipid loci
Lipid LDAK GRM
(479 SNPs)
exclude
Non-lipid LDAK GRM
(596 SNPs)
No metabolite loci (±50kb) LDAK GRM
(434,216 SNPs)
Clumping r² = 0.1
Clumping r² = 0.1
exclude
Organic acid LDAK
GRM (397 SNPs)
26,352 organic acid-SNP associations
Clumping r² = 0.1
398 organic acid loci
10,781 LD-proxies
exclude
687 non-organic acid loci
Closely-related (>0.05) LDAK GRM
(447,794 SNPs)
Non-organic acid LDAK GRM (683
SNPs)
V(G1)
V(G2)
V(G3) = h²GW-Class
V(G4) = h²GW-Notclass
∑V(G1) + V(G3) + V(G4) = h²SNP
∑V(G1) + V(G2) + V(G3) + V(G4)
= h²total
∑V(G3) + V(G4)
= h²GW-loci
V(G1)
V(G2)
V(G3) = h²GW Class
V(G4) = h²GW-Notclass
∑V(G1) + V(G3) + V(G4) = h²SNP
∑V(G1) + V(G2) + V(G3) + V(G4)
= h²total
∑V(G3) + V(G4)
= h²GW-loci
4-variance component model lipids and lipid-like molecules
4-variance component model organic acids and derivatives

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}, h^2_{SNP} and $h^2_{GW\text{-}loci}$ 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 $h^2_{GW\text{-}Class}$ and $h^2_{GW\text{-}Notclass}$ 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.

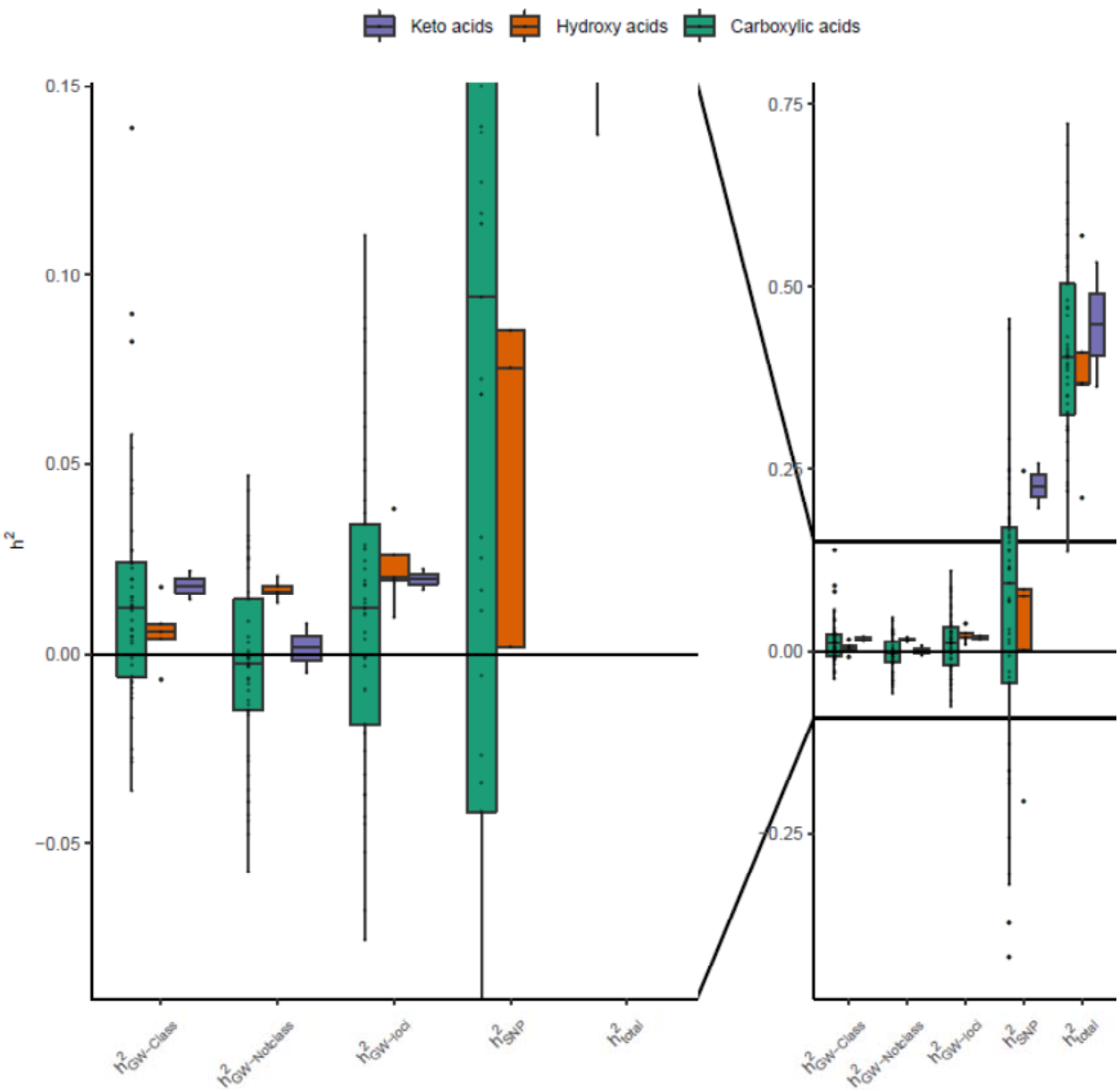
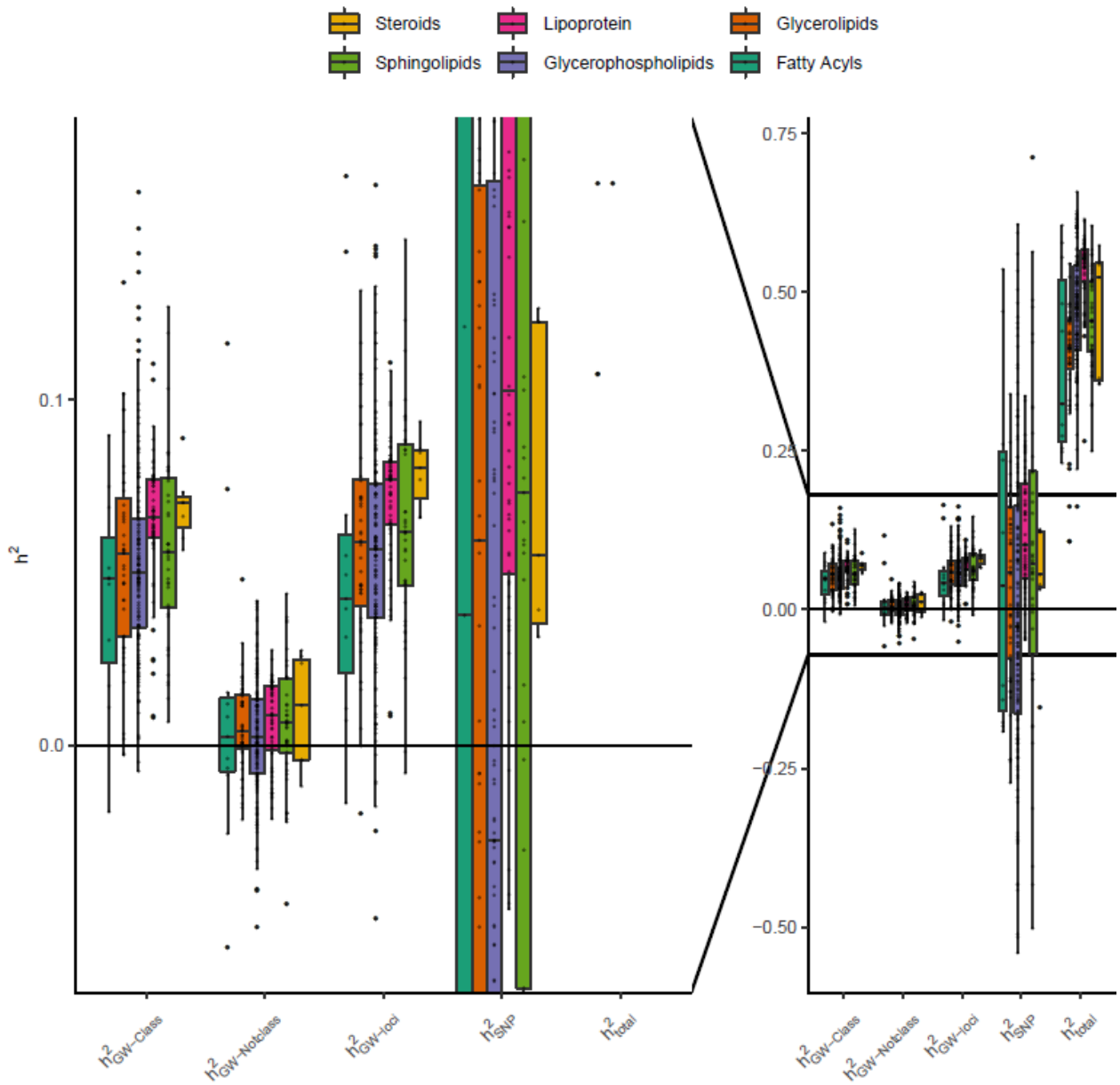

Keto acids
Hydroxy acids
Carboxylic acids
0.15
0.10
0.05
0.00
−0.05
h2
0.75
0.50
0.25
0.00
−0.25
h2GW-Class
h2GW-Noclass
h2GW-loci
h2SNP
h2total

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

Box- and dotplots of the h^2_{total}, h^2_{SNP} and $h^2_{GW\text{-}loci}$ 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 $h^2_{GW\text{-}Class}$ and $h^2_{GW\text{-}Notclass}$ 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.


Steroids
Lipoprotein
Glycerolipids
Sphingolipids
Glycerophospholipids
Fatty Acyls
h2
0.1
0.0
0.75
0.50
0.25
0.00
−0.25
−0.50
h2GW-Class
h2GW-Notclass
h2GW-loci
h2SNP
h2total

Tables

Table 1. Overview of the number of unique metabolites, for which significant SNP-metabolite associations have been published, per Human Metabolome Database[22–24] '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}), SNP heritability (h^2_{snp}), heritability based on the 479 significant metabolite loci for the 'lipids' or the 397 significant metabolite loci for the 'organic acids' ($h^2_{GW\text{-}Class}$), the 596-683 significant metabolite loci not belonging to these classes ($h^2_{GW\text{-}Notclass}$) and the total heritability explained by metabolite loci (e.g., sum of $h^2_{GW\text{-}Class}$ and $h^2_{GW\text{-}Notclass}$: $h^2_{GW\text{-}loci}$), 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.

		Lipids and lipid-like molecules		**Organic acids and derivatives**	
		estimate	**s.e.**	**estimate**	**s.e.**
	mean	0.47	0.04	0.41	0.04
$\mathbf{h^2_{total}}$	*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)
	mean	0.05	0.24	0.05	0.24
$\mathbf{h^2_{SNP}}$	*median*	0.06	0.22	0.09	0.23
	range	(-0.54 - 0.71)	(0.11 - 0.35)	(-0.42 - 0.46)	(0.11 - 0.34)
	mean	0.06	0.03	0.01	0.02
$\mathbf{h^2_{GW\text{-}loci}}$	*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)
	mean	0.06	0.02	0.01	0.02
$\mathbf{h^2_{GW\text{-}Class}}$	*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)
	mean	0.00	0.02	0.00	0.02
$\mathbf{h^2_{GW\text{-}Notclass}}$	*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}), SNP heritability (h^2_{snp}) and heritability based on the 397 significant metabolite loci for the 'organic acids' ($h^2_{GW\text{-}Class}$), the 683 significant metabolite loci not belonging to this class ($h^2_{GW\text{-}Notclass}$) and the total heritability explained by metabolite loci (e.g., sum of $h^2_{GW\text{-}Class}$ and $h^2_{GW\text{-}Notclass}$: $h^2_{GW\text{-}loci}$), 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.

		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	-0.01	0.24	0.10	0.25
$\mathbf{h^2_{SNP}}$	*median*	-0.01	0.23	0.07	0.24
	range	(-0.42 - 0.46)	(0.12 - 0.34)	(-0.18 - 0.44)	(0.12 - 0.34)
	mean	0.00	0.02	0.02	0.03
$\mathbf{h^2_{GW\text{-}loci}}$	*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
$\mathbf{h^2_{GW\text{-}Class}}$	*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
$\mathbf{h^2_{GW\text{-}Notclass}}$	*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)