

1 **Sets of Co-regulated Serum Lipids are Associated with Alzheimer Disease**
2 **Pathophysiology**

3
4 Dinesh Kumar Barupal¹, Rebecca Bailli², Sili Fan¹, Andrew J. Saykin^{3,4,5}, Peter
5 J.Meikle⁶, Matthias Arnold^{7,8}, Kwangsik Nho^{3,4}, Oliver Fiehn^{1*} and Rima Kaddurah-
6 Daouk^{9*} and for the Alzheimer's Disease Neuroimaging Initiative¹⁰ and the Alzheimer
7 Disease Metabolomics Consortium¹¹

8
9 ¹NIH-West Coast Metabolomics Center, University of California, Davis, 451 Health
10 Sciences Drive, Davis, CA 95616, United States

11 ²Rosa & Co LLC, San Carlos, CA, USA

12 ³Center for Neuroimaging, Department of Radiology and Imaging Sciences, Indiana
13 University School of Medicine, Indianapolis, IN

14 ⁴Indiana Alzheimer Disease Center, Indiana University School of Medicine,
15 Indianapolis, IN

16 ⁵Medical and Molecular Genetics Department, Indiana University School of Medicine,
17 Indianapolis, IN

18 ⁶Metabolomics Laboratory, Baker Heart and Diabetes Institute, 75 Commercial Road,
19 Melbourne, VIC 3004, Australia

20 ⁷Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München,
21 German Research Center for Environmental Health, Neuherberg, Germany

22 ⁸German Center for Diabetes Research (DZD), Neuherberg, Germany

23 ⁹Department of Psychiatry and Behavioral Sciences; Department of Medicine and the
24 Duke Institute for Brain Sciences, Duke University, Durham, NC, USA

25 ¹⁰Data used in preparation of this article were obtained from the Alzheimer's Disease
26 Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators
27 within the ADNI contributed to the design and implementation of ADNI and/or provided
28 data but did not participate in analysis or writing of this report. A complete listing of
29 ADNI investigators can be found at: [http://adni.loni.usc.edu/wp-](http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf)
30 [content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf](http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf)

31 ¹¹Data used in preparation of this article were generated by the Alzheimer's Disease
32 Metabolomics Consortium (ADMC). All authors are members of the ADMC. A complete
33 listing of ADMC investigators can be found at: [https://sites.duke.edu/adnimetab/about-](https://sites.duke.edu/adnimetab/about-us/the-team/)
34 [us/the-team/](https://sites.duke.edu/adnimetab/about-us/the-team/)

35
36 **Address correspondence to:**

37 1. Oliver Fiehn

38 Email : ofiehn@ucdavis.edu Tel: +1-530-754-8258;
39 451 East Health Science Drive, Genome Center
40 University of California, Davis. 95616 CA USA

41 2. Rima Kaddurah Daouk

42 Duke University Medical Center, Durham, NC, USA
43 Phone +1-919-684-2611
44 Email: kaddu001@mc.duke.edu

45
46
47
48

49 **ABSTRACT**

50 **INTRODUCTION:** Altered regulation of lipid metabolism in Alzheimer disease (AD) can
51 be characterized using lipidomic profiling.

52 **METHOD:** 349 serum lipids were measured in 806 participants enrolled in the
53 Alzheimer Disease Neuroimaging Initiative Phase 1 (ADNI1) cohort and analysed using
54 lipid regression models and lipid set enrichment statistics.

55 **RESULTS:** AD diagnosis was associated with 7 of 28 lipid sets of which four also
56 correlated with cognitive decline, including polyunsaturated fatty acids. CSF amyloid
57 beta A β ₁₋₄₂ correlated with glucosylceramides, lysophosphatidyl cholines and
58 unsaturated triacylglycerides; CSF total tau and brain atrophy correlated with
59 monounsaturated sphingomyelins and ceramides, in addition to EPA-containing lipids.

60 **DISCUSSION:** Lipid desaturation, elongation and acyl chain remodeling are
61 dysregulated across the spectrum of AD pathogenesis. Monounsaturated lipids were
62 important in early stages of AD, while polyunsaturated lipid metabolism was associated
63 with later stages of AD.

64 **SIGNIFICANCE**

65 Both metabolic genes and co-morbidity with metabolic diseases indicate that lipid
66 metabolism is critical in the etiology of Alzheimer's disease (AD). For 800 subjects, we
67 found that sets of blood lipids were associated with current AD-biomarkers and with AD
68 clinical symptoms. Our study highlights the role of disturbed acyl chain lipid remodelling
69 in several lipid classes. Our work has significant implications on finding a cure for AD.
70 Depending on subject age, human blood lipids may have different effects on AD
71 development. Remodelling of acyl chains needs to be studied in relation to genetic

72 variants and environmental factors. Specifically, the impact of dietary supplements and
73 drugs on lipid remodelling must be investigated.

74 **ABBREVIATIONS**

75 FA – fatty acids

76 AC – acetyl carnitine

77 PC phosphatidylcholine

78 CE – cholesteryl esters

79 SM – sphingomyelins

80 Cer – Ceramide

81 PE – phosphatidylethanolamine

82 TG – triacylglycerol

83 PI – phosphatidylinositol

84 DG – diacylglycerol

85 LPC – lysophosphatidylcholine

86 A β – Amyloid Beta

87 SPARE-AD - Spatial Pattern of Abnormality for Recognition of Early Alzheimer's
88 disease

89 LCRS – Lipid Co-Regulatory Set

90 MUFA – mono unsaturated fatty acid

91 PUFA – poly unsaturated fatty acid

92 ADASCog13 - Alzheimer's Disease Assessment Scale–Cognitive subscale

93 ADNI – Alzheimer's disease neuroimaging initiative

94 EPA – eicosapentaenoic acid

95 DHA – docosahexaenoic acid

96 CSF – cerebrospinal fluid

97 **1. Introduction**

98 The failure of clinical trials of disease modifying agents in Alzheimer's disease (AD)
99 highlights our limited knowledge about underlying pathophysiological mechanisms. AD
100 often presents with diabetes co-morbidity and a wide range of metabolic perturbations
101 occurring early in the disease process (1). APOE- ϵ 4 is by far the strongest single gene
102 variant contributing to increased AD risk and plays key roles in lipid transport and
103 metabolism. Presence of the APOE- ϵ 4 variant is correlated with higher cholesterol levels
104 in the blood (2). More than twenty additional genes have recently been implicated in AD
105 with functional roles in lipid processing, immune regulation and phagocytosis. Hence,
106 both co-morbidities and known gene variants support the idea that metabolic
107 dysfunctions may contribute to AD onset and progression.

108 Lipidomics methods using liquid chromatography and mass spectrometry (LC/MS) yield
109 reliable measurements of hundreds of lipids in biological samples. LC/MS methods have
110 been used in AD studies in attempts to define possible risk factors (3-7), diagnostic
111 markers (8) and for highlighting novel drug targets (9-11). Perturbations in ceramides
112 and related sphingomyelin metabolism (4, 7) were noted in many of these studies
113 pointing towards a possible role of these lipids in aberrant signalling pathways,
114 membrane remodelling, and apoptotic cascades during AD progression.

115 Changes in phosphatidylcholines were observed in several studies (11-13) pointing to a
116 possible role for phospholipid metabolism in AD pathogenesis. Yet, AD risk prediction
117 failed to replicate using a phosphatidylcholine (PC) biomarker panel(11, 14). Partial
118 correlation network analysis indicated early AD biomarker A β ₁₋₄₂ was associated with PC
119 and sphingomyelin (SM) (11). These studies support our hypothesis that distinct lipid

120 biochemical pathways were dysregulated in early and late phase of AD. Here, we used
121 LC-MS/MS based serum lipidomics analysis measured in the ADNI I cohort to define the
122 lipid co-regulatory network of AD phenotypes, a statistical analysis tool that previously
123 has been successfully used in the analysis of transcriptomic data (15). We investigated
124 correlation of lipid sets with (1) Disease diagnosis, (2) CSF markers of disease $A\beta_{1-42}$,
125 CSF total tau and (3) cognitive decline and brain atrophy.

126 **2. Material and methods**

127 **2.1. Study cohort**

128 Data used in the preparation of this article were obtained from the Alzheimer's Disease
129 Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in
130 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner,
131 MD. The primary goal of ADNI has been to test whether serial magnetic resonance
132 imaging (MRI), positron emission tomography (PET), other biological markers, and
133 clinical and neuropsychological assessment can be combined to measure the
134 progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). For
135 up-to-date information, see www.adni-info.org.

136 The ADNI cohort information was downloaded from the ADNI data repository
137 (<http://adni.loni.ucla.edu/>), supplying all the demographic information,
138 neuropsychological and clinical assessment data, and diagnostic information that was
139 previously published (16). Prior Institutional Review Board approval was obtained at
140 each participating institution and written informed consent was obtained for all
141 participants. Information about the ADNI project is provided on <http://www.adni-info.org/>
142 and the associated publication (17).

143 **2.2. Pathology, clinical and lipidomics data**

144 Untargeted lipidomics, AD diagnosis, CSF biomarkers including Total Tau (t-tau) and
145 amyloid beta ($A\beta_{1-42}$), cognitive decline (ADAScog13), brain atrophy represented by
146 Spatial Pattern of Abnormality for Recognition of Early Alzheimer's disease (SPARE-AD)(17)
147 data were obtained from the ADNI repository (<http://www.adni-info.org/>). Generation and
148 quality control of lipidomics data have been described in (18) .

149 **2.3. Detection of sets of co-regulated lipids**

150 A pair-wise Spearman-rank correlation matrix was generated for lipids using the R
151 function `cor.test`. The matrix was converted to a hierarchical tree model using the `hclust`
152 function in R with the ward linkage method. The resulting tree model was divided into
153 clusters using the tree cutting algorithm `dynamicTreeCut` (19). We used a minimum
154 cluster size of three and a split depth of four in the tree cutting method.

155 **2.4. Association modeling**

156 Linear regression models were established for association of serum lipid abundances
157 and CSF biomarkers and indices for cognitive decline and brain atrophy. No confounding
158 variables were included in the regression models. Logistic regression models were
159 calculated to associate serum lipids with AD diagnosis. Lipid abundances were scaled
160 by the mean subtracting approach. All models were unadjusted to identify all the lipid
161 co-regulatory sets that were associated specifically with only AD or with AD and other
162 demographics or confounding factors. Data from all ADNI-1 participants were included
163 in the analysis.

164 **2.5. Lipid set enrichment analysis**

165 Co-regulatory lipid sets detected by the `dynamicTreeCut` method (19) were used as an
166 input for cluster enrichment analysis using the Kolmogorov–Smirnov test as described
167 in the ChemRICH method (20). In this test, the distribution of p-values was assumed to
168 be uniform under a null hypothesis for a lipid cluster. Raw p-values obtained from the

169 linear and logistic models were used as input for computing the enrichment statistics by
170 comparing the experimental p-values with the uniform distribution. Set level p-values
171 were adjusted for false discovery rate using the Benjamini–Hochberg method.

172 **2.6. Source code**

173 All statistical analyses were performed in R programming language version 3.4.0.

174 The R-script is available at <http://github.com/barupal/ADNI>

175

176 **3. Results**

177 The main objective of the study was to identify lipid co-regulatory modules that were
178 associated with AD diagnosis and its clinical and pathological features. In this direction,
179 we first computed univariate association models and obtained raw p-values for each
180 lipid. Next, we identified lipid co-regulatory modules, which were then used as set-
181 definitions for a lipid-set enrichment analysis using the raw p-values for lipids. To
182 minimize the false-negative rate on the set-level statistics, univariate p-values were not
183 corrected for the multiple hypothesis testing.

184 **3.1. Subject cohort and lipidomics details**

185 Supplement Table 1 summarizes the details for the 806 ADNI participants at baseline
186 included in the present study. The baseline ADNI1 serum lipidomics dataset contained
187 16 different lipid chemical classes summarizing 349 annotated lipids (Table 1).

188 **3.2. Regression models for individual lipids**

189 We first tested all individual lipids for their association with both early and late AD
190 pathogenic markers and cognitive changes (Supplement Table S2). Raw p-values from
191 these association models will be used as an input for the lipid set enrichment analysis in
192 the following section. Figure 1 shows the number of significantly associated lipids in
193 these regression models. A total of 168 lipids were found to be significant in at least one

194 regression model (raw p -value < 0.05), making it difficult to biologically interpret them on
195 individual lipid level. Two AD phenotypes showed strong positive associations with
196 individual serum lipids, CSF Total tau and SPARE AD. Conversely, three phenotypes
197 were mostly negatively associated with individual blood lipids, including the two related
198 phenotypes AD diagnosis and its major contributor, ADASCog13. Overall, AD diagnosis
199 was associated with a decline in many lipid levels which could point to lower metabolic
200 activity in specific lipid metabolic pathways. When analyzing all individual lipids that were
201 associated with at least one AD-phenotype, we found a very high specificity of lipid
202 associations with a particular AD-phenotype (Figure 2 and Table S3). 48% (168/349) of
203 all lipids were associated with at least one AD-phenotype (Table S3). Specifically, for
204 known lipids, 63% of all AD-phenotype associated lipids were specific to only one
205 phenotype and not shared with others (Figure 3). 28 % of the detected associations of
206 known lipids were shared between two phenotypes, driven by lipids shared between the
207 two related phenotypes AD diagnosis and its major contributor, ADASCog13, in addition
208 to lipids shared between total tau and SPARE-AD. Conversely, Abeta142 showed few
209 shared lipids. Importantly, there was no identified lipid that was shared between four
210 phenotypes, and only one lipid that was associated with all AD-phenotypes (arachidonyl-
211 lysophosphatidylethanolamine; LPE C20:4). Many lipids are co-regulated by the activity
212 of specific lipases or other enzymes involved in lipid remodeling. Identifying
213 commonalities of biochemical mechanisms may lead to underlying genetic drivers or
214 environmental factors implicated in AD-etiology. Therefore, we next focused on
215 identifying sets of co-regulated lipids associated with AD pathophysiology rather than
216 interpreting individual lipids.

217 **3.3. Identifying sets of co-regulated lipids**

218 Lipid classification often uses chemical structure as the only criterion. To specify the
219 biochemical relationships between circulating blood lipids, we instead used correlation
220 analysis to determine sets of lipids. A pair-wise Spearman correlation matrix followed by
221 hierarchical clustering with the DynamicTreeCut dendrogram cutting method (19) yielded
222 a total of 28 co-regulated lipid sets in the ADNI1 dataset (Figure 3). The mean size was
223 12.5 lipids per set, ranging from 4 to 28 members. These lipid sets (LM) were named
224 LM1 to LM28. The average Spearman correlation coefficient ρ across sets was 0.63
225 with a range of $0.19 < \rho < 0.82$. Figure 3 and Supplemental Table S2 show that some
226 lipid co-regulatory sets were composed of lipids from the same chemical classes (such
227 as Set-17 for free fatty acids, Set-1 for triacylglycerides and Set-14 for ceramides)
228 whereas other sets were heterogeneous (such as Set-3 consisting of ceramides and
229 sphingomyelins, or Set-7 that includes phosphatidylinositols and phosphatidylcholines).
230 Interestingly, several classes of lipids were found with distinct co-regulation within each
231 class. For example, triacylglycerides were not found as one large group of co-regulated
232 species, but clustered in three specific sets, and similarly, free fatty acids were found in
233 two different sets, Set-9 consisting only of saturated fatty acids and Set-17 comprised
234 only of unsaturated fatty acids. Similarly, other lipid classes were distributed across
235 different sets, too. For example, phosphatidylcholines were found in five sets and
236 sphingomyelins were co-regulated in three sets, indicating downstream regulation of lipid
237 biochemistry by specific elongases, desaturases, lipases, acyl-transferases within each
238 lipid class (Figure 2).

239 **3.4 Associating lipid sets with AD-phenotypes**

240 These lipid groups served as input for a lipid-set enrichment analysis (LSEA)(20) along
241 with the p-value and beta coefficient results from the regression models. Overall, 19 out
242 of 28 lipid sets were significantly associated with at least one AD-phenotype (Figure 4,

243 Table S3) using the Kolmogorov-Smirnoff statistical test with FDR-corrections. Eight sets
244 were uniquely associated with only one specific AD-phenotype, but only one set was
245 associated with four phenotypes, Set-11, consisting primarily of ceramides and
246 phosphatidylcholines. Set-11 did not include polyunsaturated acyl chains with three or
247 more double bonds. Six sets were associated with two AD-phenotypes and four sets
248 were correlated with three AD-phenotypes, but no set correlated with all five phenotypes.
249 More than two-thirds of all associations were positively correlated between lipid sets and
250 phenotypes, mostly driven by the t-tau phenotype that also had the highest number of
251 correlated lipid sets. Conversely, ADASCog13 showed the highest number of negative
252 associations with lipid sets. We therefore investigated the individual phenotypes with
253 respect to the composition of their associated lipid sets.

254 **3.4.1. Lipid sets associated with AD diagnosis**

255 AD-diagnosis was significantly associated with seven distinct lipid sets (Figure 4) after
256 FDR correction. Specifically, the phenotype was highly significantly negatively correlated
257 with lipid Set-26 and Set-4. Both sets were comprised of acyl chains with at least one
258 polyunsaturated fatty acyl chain (PUFA) (see Table S2), either eicosapentaenoic acid
259 (EPA), docosahexaenoic acid (DHA) or arachidonic acid (AA). Set-26 consisted
260 exclusively of triacylglycerides that also contained either EPA or DHA, but not a single
261 saturated fatty acid. Set-4 was mixed between different phospholipid head groups,
262 cholesteryl esters and free fatty acids, indicating that the co-regulation mechanisms
263 focused on the modulation and incorporation of acyl chains irrespective of the lipid class.
264 Set-23 was also negatively correlated with AD-diagnosis and comprised of DHA-
265 containing choline- and ethanolamine-plasmalogens. Conversely, two other sets of lipids
266 were positively associated with AD-diagnosis, most significantly for Set-5 and Set-20,
267 and less significantly with Set-11 and Set-12. Set-5 contained co-regulated di- and

268 triacylglycerols with acyl groups that did not contain any PUFA with four or more double
269 bonds, and only one single lipid with linolenic acid (C18:3). Set-20 was exclusively
270 composed of unsaturated choline-plasmalogens, but not containing any EPA or DHA
271 acyl chain.

272

273 **3.4.2 Lipid sets associated with CSF A β ₁₋₄₂**

274 CSF A β ₁₋₄₂ was significantly associated with four lipid sets (Figure 4). Three sets were
275 negatively correlated, Set-11, Set-7 and Set-8. Set-7 was the only lipid set that contained
276 phosphatidylinositols, in addition to phosphatidyl cholines. Acyl chains were primarily
277 saturated or mono- and di-unsaturated. Similarly, Set-8, consisted mostly of desaturated
278 acyl groups with less than four double bonds, exclusively found as lyso-
279 phosphatidylcholines. In the same way, no PUFA-acyl chains were found in Set-11, a
280 heterogenous set of ceramides and choline-plasmalogens. Importantly, the only positive
281 association of a lipid set with CSF A β ₁₋₄₂ was Set-26 that was completely made of PUFA-
282 triacylglycerides.

283

284 **3.4.3 Lipid sets associated with CSF tau**

285 CSF total tau correlated with 12 lipid sets, the highest number of associated lipid sets
286 among all phenotypes (Figure 4). All sets except Set-1 were positively correlated with
287 CSF-total tau. Three unique sets that were not shared with other phenotypes. Set-16
288 was composed of acylcarnitines with increasing degree of desaturation, and Set-17 was
289 a set of monounsaturated fatty acids. Set-1 was less significant in comparison to other
290 set associations. Four sets were shared with brain atrophy, four sets were shared with
291 AD-diagnosis, two sets with amyloid beta and four lipid sets were shared with cognitive
292 decline. Notably, set-19 was also associated with brain atrophy and contained mostly

293 EPA-side chain phosphatidylcholines. Set-3 was composed of sphingomyelins and
294 ceramides that were not associated with diagnosis or amyloid beta, but instead was also
295 linked with cognitive decline and SPARE-AD.

296

297 **3.4.4 Lipid sets associated with Brain atrophy (SPARE-AD)**

298 Brain atrophy was most significantly associated with Set 27, 19, 11 and 2 (Figure 4).
299 Three sets (Set 2, Set 11, Set 27) were void of PUFA-side chains with either
300 phospholipid or sphingolipid head groups. Conversely, Set 19 contained mostly EPA-
301 side chain phosphatidylcholines and was further associated with CSF total tau. Similarly,
302 Set 21 was associated with both phenotypes, containing phospholipids and their lyso-
303 forms with the PUFA acyl chain arachidonic acid.

304

305 **3.4.5 Lipid sets associated with cognitive functions**

306 Most of the lipid sets associated with cognitive decline were also associated with AD
307 diagnosis (Figure 4). Additionally, it was negatively associated with set Set-22 which
308 consisted of ethanolamine-plasmalogens.

309

310 **4. Discussion**

311 We here focused on associations between blood lipids and five AD-phenotypes guided
312 by known contributions of lipids and metabolic co-morbidities to Alzheimer's disease. We
313 systematically tested both the association of individual lipids and the association with
314 sets of co-regulated lipids. This approach showed an important advantages over
315 previous "feature" based lipidomics-AD studies (9), (21) that did not focus on specific
316 lipid groups, their side chains or their biological regulation. Without clear lipid
317 identification, feature-based associations miss biological insights and have a high risk of

318 not being validated in subsequent studies because each individual lipid may cause more
319 than one feature in LC-MS based lipidomics(21, 22). Instead we used the largest
320 published AD-lipidomics data set (18) to date with 349 identified lipids belonging to 13
321 major lipid classes, identified by extensive MS/MS fragmentation analysis (23) and
322 enabling analyses reaching to the level of acyl chains. A second difference to previous
323 efforts was a focus on summarizing lipids by statistical co-regulation instead of only
324 relying on univariate analysis or grouping by lipid head groups. This expansion of classic
325 statistical analysis was critical to extend from diagnostic biomarkers (that need
326 correction for multiple testing using false discovery rate (FDR) adjustments) to revealing
327 underlying biological mechanisms. The axiom of univariate analyses, the mutual
328 independence of variables, is untrue in lipid biology. Moreover, stringent FDR corrections
329 lead to an increased number of false negative results and compromise the statistical
330 power to investigate the biological mechanisms and pathways. As lipids are poorly
331 presented in biochemical pathway databases (20), classic metabolic pathway
332 enrichment analysis (24) ignores a majority of detected lipids and is unsuitable for
333 lipidomics. Instead, Spearman-rank correlation based matrices yielded specific clusters
334 of lipids associated with Alzheimer's disease phenotypes by using the robust
335 Kolmogorov-Smirnov test for p -value distributions. These lipid sets showed very distinct
336 metabolic features that we identified as preferential use of specific polyunsaturated fatty
337 acids that replaced saturated or monounsaturated fatty acids for distinct lipid classes.
338 These mechanisms of lipid desaturation, elongation, and acyl-chain remodeling were
339 disturbed in early and later stages of Alzheimer disease. A minimal overlap among lipid
340 sets was observed (Figure 3) with respect to statistical associations with AD phenotypes,
341 indicating that quite distinct lipid biochemical processes were involved in the early and
342 later stages of AD. Lipid metabolic pathways associated with the early stage, in

343 particular, may provide new therapeutic targets to stop AD progression. MUFA-
344 containing lipids were positively associated with the brain atrophy and tau accumulation
345 whereas PUFA-containing lipids were negatively associated with AD diagnosis and
346 cognitive decline. Therapeutic strategies targeting MUFA lipid pathways at the early
347 stages of AD could therefore be potentially more effective in delaying the progression of
348 the disease.

349 **4.1 Lipids linked to the amyloid beta clearance pathway**

350 A decrease in the **CSF A β ₁₋₄₂** peptide marker is indicating a poor clearance of the peptide
351 in the brain, leading to its extra-neuronal accumulation. In our study, poor clearance was
352 indicated by negative associations with lipids sets, including sets that contained
353 phosphatidylinositols, lysoPCs, ceramides and choline-plasmalogens and PUFA TGs.
354 The amyloid β peptide is known to cause mitochondrial dysfunction (25) which can lead
355 to neurodegeneration via autophagic cascades (26, 27). The associated lipids,
356 specifically ceramides and phosphatidylinositols and lysoPCs have been linked with cell
357 death and may also contribute in the Amyloid Beta mediated toxicity in neurons (28-30).
358 Higher levels of ceramides containing oleic acid (C18:1) have been shown to increase
359 AD risk (4, 5). We validate this finding in our study and also observed lower levels of
360 phosphoinositols containing polyunsaturated fatty acids to correlate with poor Amyloid-
361 beta clearance. An alternative explanation to our data is an impaired amyloid beta
362 clearance in the liver(31)that subsequently leads to dysregulation of lipid metabolism in
363 the liver. Overall, our data suggest that these lipid sets can serve as serum biomarkers
364 for disturbed Amyloid beta pathway regulation in brain and can complement Amyloid
365 beta imaging assays.

366

367 **4.2 Cerebrospinal fluid total tau**

368 CSF tau is a marker for accumulating tau tangles in the brain tissues, causing
369 neurodegeneration. We found that the total CSF tau marker was significantly associated
370 with lipid sets enriched in monounsaturated fatty acids, acyl-carnitines, ceramides,
371 sphingomyelins, and EPA containing phosphatidylcholines. Increased fatty acids and
372 acyl-carnitines are known markers of impaired fatty acid beta oxidation in
373 mitochondria(32), specially during metabolic diseases such as diabetes and obesity(33,
374 34). We found free fatty acids and acylcarnitines to be positively correlated with total tau,
375 supporting the notion of tau mediated neurodegeneration and mitochondrial
376 dysfunctions. Mitochondrial impairment was further evidenced by positive associations
377 of total tau with sets of ceramides, because accumulating ceramide are known to induce
378 cell death and to increase the AD risk in normal subjects (5) Rozen et al. 2011. Higher
379 ceramide levels were also reported for early stage Alzheimer's disease (35-37). These
380 findings indicate that these lipids may be involved in early neurodegenerative pathways,
381 and their underlying pathways might lead to candidates for new therapeutic strategies.

382

383 **4.3 Lipid sets linked with brain atrophy**

384 SPARE-AD is a composite index of brain atrophy and indicates the neurodegeneration
385 magnitude. We found a high overlap of lipid sets that were associated with both SPARE-AD and
386 total tau, reinforcing the usability of these serum lipids as biomarkers for neurodegeneration.
387 These lipid sets included phosphatidylcholines and sphingolipids that were enriched in
388 polyunsaturated fatty acyls (PUFA) eicosapentaenoic acid and arachidonic acid (EPA, AA).
389 These fatty acids are main components of brain lipids(38, 39). The loss of brain tissue may cause
390 an increase in levels of serum lipids that include EPA and AA as acyl groups through lipid
391 remodelling (40, 41). We here identify lipid pathways associated with tau-mediated brain atrophy
392 that eventually contributes to AD.

393

394 **4.4 AD diagnosis and cognitive decline**

395 Previous publications reported that lower levels of PUFA in AD subjects across multiple
396 lipid classes, along with higher levels of monounsaturated lipids (4, 8, 9, 42-46). We
397 found numerous, very significant associations of omega-3 and omega-6 containing
398 complex lipids with AD diagnosis and cognitive functions. Our analysis is consistent with
399 these results as shown by AD associated lipids in Set-4, Set-20, Set-23 (Figure 4). We
400 here specify that the major difference is not related to total levels of mono- or
401 polyunsaturated fatty acids, but the extent at which these fatty acids are incorporated
402 into different complex lipids. Clear differences in circulating PUFA phospholipid and
403 PUFA triacylglycerol levels in AD subjects in comparison to normal subjects were
404 observed, likely due to dysregulation of biosynthesis in liver. Here, substrate preference
405 of MGAT and DGAT enzymes in the liver may play an important role, but the exact
406 specificities of acyltransferase enzymes (and their corresponding lipase enzymes) are
407 not well studied. Levels of anti-inflammatory plasmalogens (47), important lipids for brain
408 membrane functions (45, 48), were decreased in AD patients in comparison to
409 cognitively normal subjects. Lower levels of plasmalogens have been linked to AD (45).
410 However, in clinical trials, EPA and DHA supplementation do not improve the cognitive
411 function of AD subjects (49). Nutritional intervention trials such as the European
412 LipiDiDiet have failed to show any cognitive improvement in AD subjects. A broad-
413 spectrum effect of FOS on additional lipid pathways may explain the failure of this trial
414 and warrants further lipidomics studies for serum specimens of this trial's participants. It
415 was observed that the incorporation of omega-6 fatty acids was increased in AD
416 subjects. These fatty acids are well-known precursors to pro-inflammatory molecules
417 such as leukotrienes. Further studies are needed to test if post-mortem brain tissues of

418 AD subjects show similar disruption in fatty acid incorporation and if these patterns
419 correlate with AD severity or other AD phenotypes.

420

421 **5. Conclusions**

422 Using co-regulated sets of lipids enabled us to find significant associations of lipids with
423 Alzheimer's disease that led to biochemical mechanisms. Across the spectrum of AD
424 progression, pathways were dysregulated that pointed to lipid desaturation, elongation
425 and remodelling. These pathways provide new targets as well candidate biomarkers for
426 the population screening for AD prevention. Future studies are needed to tease out the
427 roles of genetic variations, drug, and diet the metabolism of MUFA and PUFAs and their
428 complex lipids and their roles in AD.

429

430 **Acknowledgments**

431 **Funding**

432 This work was funded through NIH awards U54 AI138370, U19 AG023122 and U2C ES030158.
433 National Institute on Aging (R01AG046171, RF1AG051550, and RF1AG057452 and
434 3U01AG024904-09S4) supported the Alzheimer Disease Metabolomics Consortium which is a
435 part of NIA national initiatives AMP-AD and M2OVE AD. Data collection and sharing for this
436 project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National
437 Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award
438 number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National
439 Institute of Biomedical Imaging and Bioengineering, and through generous contributions from
440 the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon
441 Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai
442 Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd
443 and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen

444 Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical
445 Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics,
446 LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation;
447 Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition
448 Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI
449 clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the
450 National Institutes of Health (www.fnih.org). The grantee organization is the Northern California
451 Institute for Research and Education, and the study is coordinated by the Alzheimer's
452 Therapeutic Research Institute at the University of Southern California. ADNI data are
453 disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

454 **Author's contribution**

455 DKB and OF generated the lipidomics dataset. DKB, RB, RKD and OF design the study.
456 DKB and SF performed the statistical analyses. AJS, PJM, MA and KN contributed in
457 data interpretation. All author contributed in the manuscript writing.

458 Transparency declaration: Authors affirms that the manuscript is an honest, accurate,
459 and transparent account of the study being reported; no important aspects of the study
460 have been omitted; and any discrepancies from the study as planned have been
461 explained.

462

463 **Figure legends**

464 **Figure 1. Co-regulated sets of serum lipids in the ADNI lipidomics dataset. Sets**

465 were detected by the dynamicTreeCut algorithm (see method). Node colors show

466 different chemical classes. FA – fatty acids, AC – acyl carnitines, PC

467 phosphatidylcholines, CE – cholesteryl esters, SM – sphingomyelins, Cer – ceramides,

468 PE – phosphatidylethanolamines, TG – triacylglycerols, PI – phosphatidylinositols, DG

469 – diacylglycerols, LPC – lysophosphatidylcholines.

470 **Figure 2. Number of significantly different lipids with AD phenotypes in**

471 **univariate statistics.** Directions of beta coefficients in regression models are given by

472 colors as blue (negative) and red (positive) associations using uncorrected $p < 0.05$

473 values. CN : cognitively normal, LMCI : late mild cognitive impairment, AD : Alzheimer's

474 disease. DIAG : linear models for diagnosis, tau – linear model for tau, $A\beta_{1-42}$ – linear

475 model for amyloid beta peptide 42, SPARE-AD – linear model for Spatial Pattern of

476 Abnormality for Recognition of Early Alzheimer's disease index, ADASCog13 -

477 Cognitive Subscale of the Alzheimer's Disease Assessment Scale index.

478

479 **Figure 3. Number of significantly associated lipids across AD-phenotypes.**

480 Uncorrected $p < 0.05$ values for five AD-phenotypes. DIAG : linear models for

481 diagnosis, tau – linear model for tau, ABETA142 – linear model for amyloid beta

482 peptide 42, SPARE-AD – linear model for Spatial Pattern of Abnormality for

483 Recognition of Early Alzheimer's disease index, ADASCog13 - Cognitive Subscale of

484 the Alzheimer's Disease Assessment Scale index.

485 **Figure 4. Co-regulated sets of lipids significantly associated with AD-**
486 **phenotypes.** Direction of associations is given by red (positive) and blue (negative)
487 edge colors. Line thickness indicates the significance of associations (see
488 Supplementary Table 4 for details). Lipid compositions for each set are shown in
489 Figure 1 and Supplementary Table S1. DIAG : linear models for diagnosis, tau – linear
490 model for tau, ABETA142 – linear model for amyloid beta peptide 42, SPARE-AD –
491 linear model for Spatial Pattern of Abnormality for Recognition of Early Alzheimer’s
492 disease index, ADASCog13 - Cognitive Subscale of the Alzheimer’s Disease
493 Assessment Scale index.

494

495 **Tables**

496 **Table 1. Lipid classes and sub-classes in the ADNI serum lipidomics untargeted**
497 **dataset**

Class	Subclass	Count
Acylcarnitine (AC)	Acylcarnitine	9
Free fatty acid (FA)	Fatty acid	29
Sterol lipids	Cholesterol	1
	Cholesteroyl ester (CE)	8
Phospholipid	Lysophosphatidylcholine (LPC)	22
	Lysophosphatidylethanolamine (LPE)	4
	Phosphatidylcholine (PC)	53
	Phosphatidylethanolamine (PE)	11
	Phosphatidylinositol (PI)	11
	Plasmalogen phosphatidylcholine (p-PC)	28
	Plasmalogen phosphatidylethanolamine (p-PE)	15
Sphingolipid	Ceramide (CER)	19
	Glucosylceramide (GluCer)	8
	Sphingomyelin (SM)	34
Acylglycerols	Diacylglycerol (DG)	13
	Triacylglycerol (TG)	84

498

499

500

501 References

- 502 1. Scheltens P, *et al.* (2016) Alzheimer's disease. *Lancet* 388(10043):505-517.
- 503 2. Liu CC, Liu CC, Kanekiyo T, Xu H, & Bu G (2013) Apolipoprotein E and Alzheimer disease: risk,
504 mechanisms and therapy. *Nature reviews. Neurology* 9(2):106-118.
- 505 3. Bressler J, *et al.* (2017) Metabolomics and cognition in African American adults in midlife: the
506 atherosclerosis risk in communities study. *Translational psychiatry* 7(7):e1173.
- 507 4. Han X, *et al.* (2011) Metabolomics in early Alzheimer's disease: identification of altered plasma
508 sphingolipidome using shotgun lipidomics. *PLoS one* 6(7):e21643.
- 509 5. Mielke MM, *et al.* (2012) Serum ceramides increase the risk of Alzheimer disease: the Women's
510 Health and Aging Study II. *Neurology* 79(7):633-641.
- 511 6. Schilling S, *et al.* (2017) Differential associations of plasma lipids with incident dementia and
512 dementia subtypes in the 3C Study: A longitudinal, population-based prospective cohort study.
513 *PLoS medicine* 14(3):e1002265.
- 514 7. van der Lee SJ, *et al.* (2018) Circulating metabolites and general cognitive ability and dementia:
515 Evidence from 11 cohort studies. *Alzheimer's & dementia : the journal of the Alzheimer's*
516 *Association* 14(6):707-722.
- 517 8. Proitsi P, *et al.* (2017) Association of blood lipids with Alzheimer's disease: A comprehensive
518 lipidomics analysis. *Alzheimer's & dementia : the journal of the Alzheimer's Association*
519 13(2):140-151.
- 520 9. Proitsi P, *et al.* (2015) Plasma lipidomics analysis finds long chain cholesteryl esters to be
521 associated with Alzheimer's disease. *Translational psychiatry* 5:e494.
- 522 10. Varma VR, *et al.* (2018) Brain and blood metabolite signatures of pathology and progression in
523 Alzheimer disease: A targeted metabolomics study. *PLoS medicine* 15(1):e1002482.
- 524 11. Toledo JB, *et al.* (2017) Metabolic network failures in Alzheimer's disease: A biochemical road
525 map. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 13(9):965-984.
- 526 12. Mapstone M, *et al.* (2014) Plasma phospholipids identify antecedent memory impairment in
527 older adults. *Nature medicine* 20(4):415-418.
- 528 13. Fiandaca MS, *et al.* (2015) Plasma 24-metabolite Panel Predicts Preclinical Transition to Clinical
529 Stages of Alzheimer's Disease. *Front Neurol* 6:237.
- 530 14. Casanova R, *et al.* (2016) Blood metabolite markers of preclinical Alzheimer's disease in two
531 longitudinally followed cohorts of older individuals. *Alzheimer's & dementia : the journal of the*
532 *Alzheimer's Association* 12(7):815-822.
- 533 15. Xu M, *et al.* (2018) A systematic integrated analysis of brain expression profiles reveals YAP1
534 and other prioritized hub genes as important upstream regulators in Alzheimer's disease.
535 *Alzheimer's & dementia : the journal of the Alzheimer's Association* 14(2):215-229.
- 536 16. Toledo JB, *et al.* (2017) Metabolic network failures in Alzheimer's disease-A biochemical road
537 map. *Alzheimer's & dementia : the journal of the Alzheimer's Association*.
- 538 17. Toledo JB, *et al.* (2013) Relationship between plasma analytes and SPARE-AD defined brain
539 atrophy patterns in ADNI. *PLoS one* 8(2):e55531.
- 540 18. Barupal DK, *et al.* (2018) Generation and quality control of lipidomics data for the alzheimer's
541 disease neuroimaging initiative cohort. *Sci Data* 5:180263.
- 542 19. Langfelder P, Zhang B, & Horvath S (2008) Defining clusters from a hierarchical cluster tree: the
543 Dynamic Tree Cut package for R. *Bioinformatics* 24(5):719-720.
- 544 20. Barupal DK & Fiehn O (2017) Chemical Similarity Enrichment Analysis (ChemRICH) as
545 alternative to biochemical pathway mapping for metabolomic datasets. *Sci Rep* 7(1):14567.
- 546 21. Mahieu NG & Patti GJ (2017) Systems-Level Annotation of a Metabolomics Data Set Reduces
547 25000 Features to Fewer than 1000 Unique Metabolites. *Analytical chemistry* 89(19):10397-
548 10406.

- 549 22. Bowden JA, *et al.* (2017) Harmonizing lipidomics: NIST interlaboratory comparison exercise for
550 lipidomics using SRM 1950-Metabolites in Frozen Human Plasma. *Journal of lipid research*
551 58(12):2275-2288.
- 552 23. Kind T, *et al.* (2013) LipidBlast in silico tandem mass spectrometry database for lipid
553 identification. *Nat Methods* 10(8):755-758.
- 554 24. Xia J & Wishart DS (2010) MetPA: a web-based metabolomics tool for pathway analysis and
555 visualization. *Bioinformatics* 26(18):2342-2344.
- 556 25. Mossmann D, *et al.* (2014) Amyloid-beta peptide induces mitochondrial dysfunction by
557 inhibition of preprotein maturation. *Cell metabolism* 20(4):662-669.
- 558 26. Lin MT & Beal MF (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative
559 diseases. *Nature* 443(7113):787-795.
- 560 27. Suomalainen A & Battersby BJ (2017) Mitochondrial diseases: the contribution of organelle
561 stress responses to pathology. *Nature reviews. Molecular cell biology*.
- 562 28. Elliott MR & Ravichandran KS (2016) The Dynamics of Apoptotic Cell Clearance. *Dev Cell*
563 38(2):147-160.
- 564 29. Magtanong L, Ko PJ, & Dixon SJ (2016) Emerging roles for lipids in non-apoptotic cell death. *Cell*
565 *Death Differ* 23(7):1099-1109.
- 566 30. Wong MW, *et al.* (2017) Dysregulation of lipids in Alzheimer's disease and their role as
567 potential biomarkers. *Alzheimer's & dementia : the journal of the Alzheimer's Association*.
- 568 31. Maarouf CL, *et al.* (2018) Impaired hepatic amyloid-beta degradation in Alzheimer's disease.
569 *PloS one* 13(9):e0203659.
- 570 32. Ciavardelli D, *et al.* (2016) Medium-chain plasma acylcarnitines, ketone levels, cognition, and
571 gray matter volumes in healthy elderly, mildly cognitively impaired, or Alzheimer's disease
572 subjects. *Neurobiology of aging* 43:1-12.
- 573 33. Adams SH, *et al.* (2009) Plasma acylcarnitine profiles suggest incomplete long-chain fatty acid
574 beta-oxidation and altered tricarboxylic acid cycle activity in type 2 diabetic African-American
575 women. *J Nutr* 139(6):1073-1081.
- 576 34. Serra D, Mera P, Malandrino MI, Mir JF, & Herrero L (2013) Mitochondrial fatty acid oxidation
577 in obesity. *Antioxid Redox Signal* 19(3):269-284.
- 578 35. Han X, D MH, McKeel DW, Jr., Kelley J, & Morris JC (2002) Substantial sulfatide deficiency and
579 ceramide elevation in very early Alzheimer's disease: potential role in disease pathogenesis.
580 *Journal of neurochemistry* 82(4):809-818.
- 581 36. He X, Huang Y, Li B, Gong CX, & Schuchman EH (2010) Deregulation of sphingolipid metabolism
582 in Alzheimer's disease. *Neurobiology of aging* 31(3):398-408.
- 583 37. Cutler RG, *et al.* (2004) Involvement of oxidative stress-induced abnormalities in ceramide and
584 cholesterol metabolism in brain aging and Alzheimer's disease. *Proceedings of the National*
585 *Academy of Sciences of the United States of America* 101(7):2070-2075.
- 586 38. Dyllal SC (2015) Long-chain omega-3 fatty acids and the brain: a review of the independent and
587 shared effects of EPA, DPA and DHA. *Front Aging Neurosci* 7:52.
- 588 39. Martin V, *et al.* (2010) Lipid alterations in lipid rafts from Alzheimer's disease human brain
589 cortex. *Journal of Alzheimer's disease : JAD* 19(2):489-502.
- 590 40. Hashidate-Yoshida T, *et al.* (2015) Fatty acid remodeling by LPCAT3 enriches arachidonate in
591 phospholipid membranes and regulates triglyceride transport. *Elife* 4.
- 592 41. Wang B, *et al.* (2016) Intestinal Phospholipid Remodeling Is Required for Dietary-Lipid Uptake
593 and Survival on a High-Fat Diet. *Cell metabolism* 23(3):492-504.
- 594 42. Iuliano L, *et al.* (2013) Plasma fatty acid lipidomics in amnesic mild cognitive impairment and
595 Alzheimer's disease. *Journal of Alzheimer's disease : JAD* 36(3):545-553.
- 596 43. Cunnane SC, *et al.* (2012) Plasma and brain fatty acid profiles in mild cognitive impairment and
597 Alzheimer's disease. *Journal of Alzheimer's disease : JAD* 29(3):691-697.

- 598 44. Conquer JA, Tierney MC, Zecevic J, Bettger WJ, & Fisher RH (2000) Fatty acid analysis of blood
599 plasma of patients with Alzheimer's disease, other types of dementia, and cognitive
600 impairment. *Lipids* 35(12):1305-1312.
- 601 45. Wood PL, *et al.* (2010) Circulating plasmalogen levels and Alzheimer Disease Assessment Scale-
602 Cognitive scores in Alzheimer patients. *J Psychiatry Neurosci* 35(1):59-62.
- 603 46. Mielke MM, *et al.* (2010) Plasma ceramides are altered in mild cognitive impairment and
604 predict cognitive decline and hippocampal volume loss. *Alzheimer's & dementia : the journal of*
605 *the Alzheimer's Association* 6(5):378-385.
- 606 47. Rasmiena AA, *et al.* (2015) Plasmalogen modulation attenuates atherosclerosis in ApoE- and
607 ApoE/GPx1-deficient mice. *Atherosclerosis* 243(2):598-608.
- 608 48. Scott BL & Bazan NG (1989) Membrane docosahexaenoate is supplied to the developing brain
609 and retina by the liver. *Proceedings of the National Academy of Sciences of the United States of*
610 *America* 86(8):2903-2907.
- 611 49. Mazereeuw G, Lanctot KL, Chau SA, Swardfager W, & Herrmann N (2012) Effects of omega-3
612 fatty acids on cognitive performance: a meta-analysis. *Neurobiology of aging* 33(7):1482 e1417-
613 1429.
- 614

Figure 1

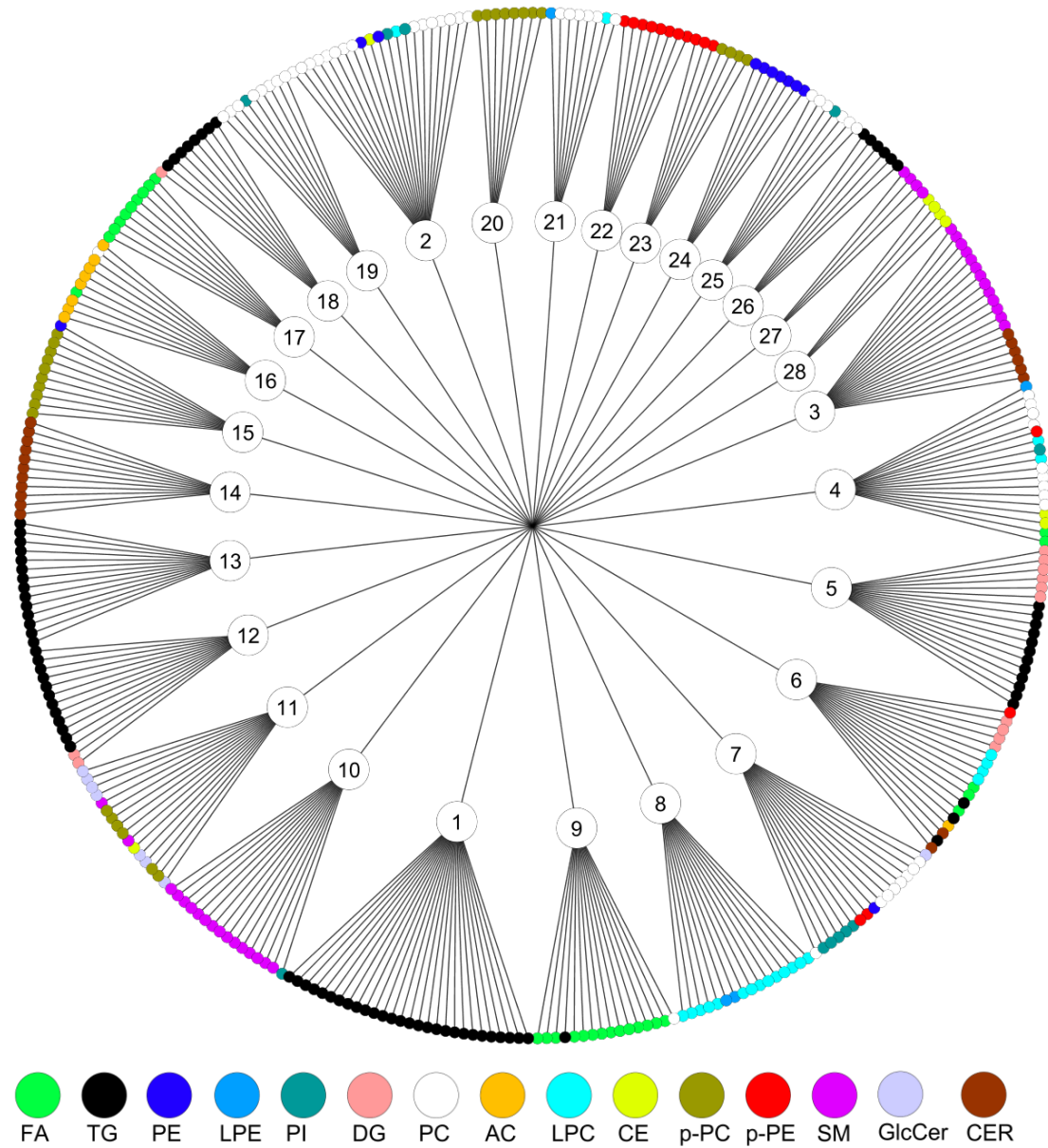


Figure 2

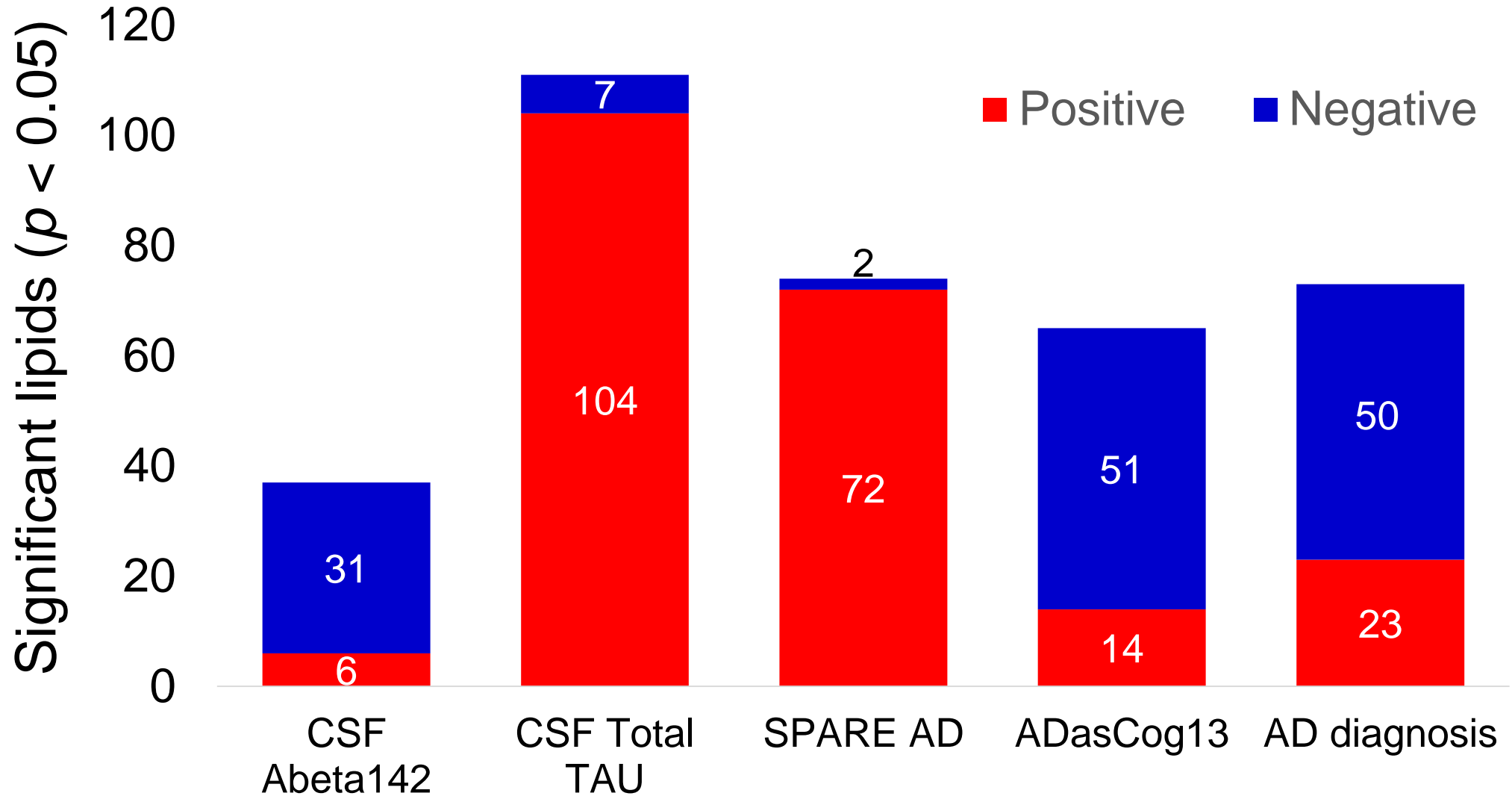


Figure 3

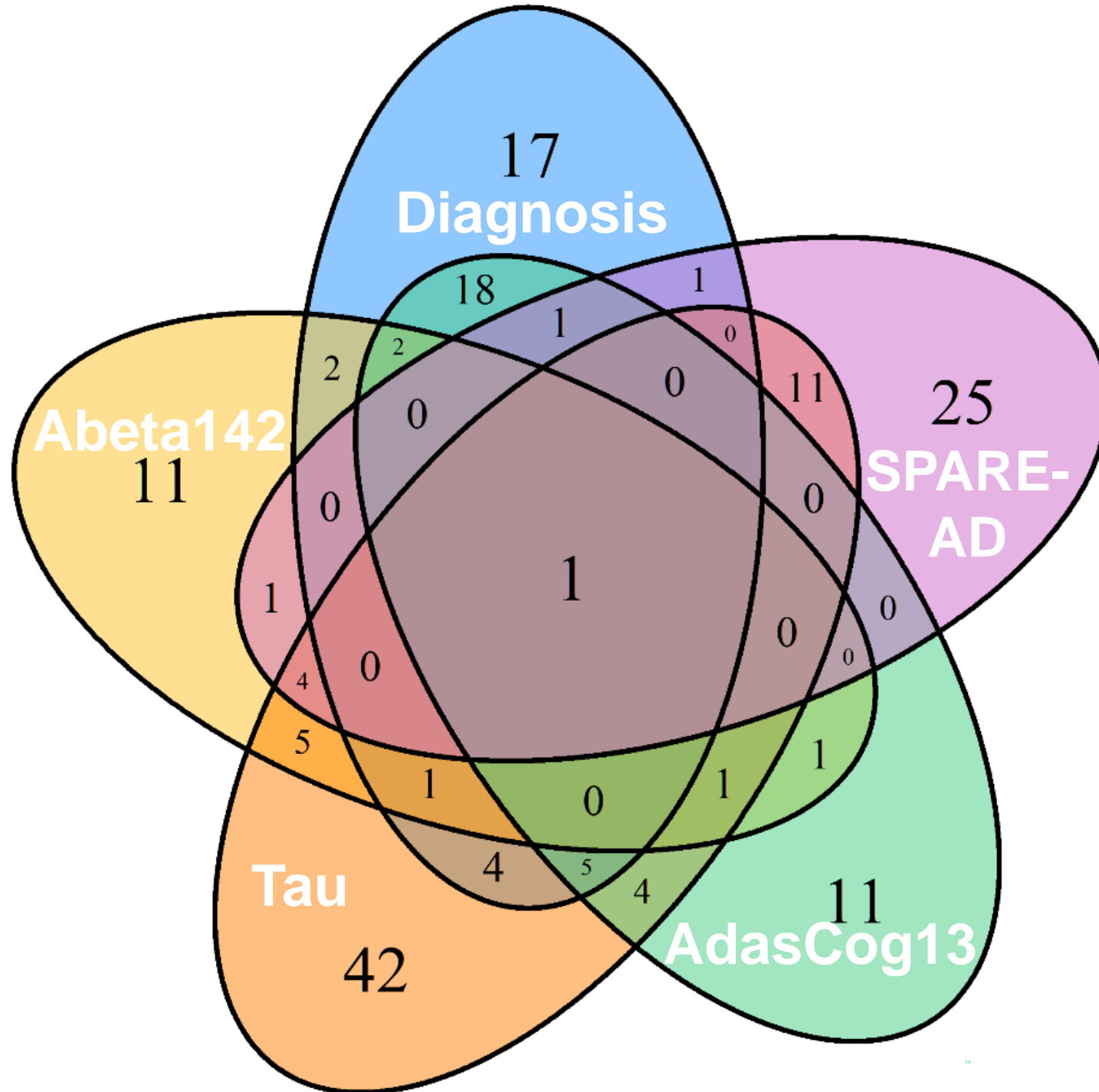


Figure 4

