Exploration of plasma lipids in mild cognitive impairment due to Alzheimer's disease

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Abstract

Background: Lipids have important structural roles in cell membranes and changes to these membrane lipids may influence β- and γ-secretase activities and thus contribute to Alzheimer's disease (AD) pathology.

Objective: To explore baseline plasma lipid profiling in participants with mild cognitive impairment (MCI) with and without AD pathology.

Method: We analysed 261 plasma lipid profiles using reversed phase chromatography mass spectrometry in cerebrospinal fluid (CSF) amyloid positive (Aβ+) or negative (Aβ-) participants with mild cognitive impairment (MCI) as compared to healthy controls. Additionally, we analysed the associations of plasma lipid profiles with performance on neuropsychological tests at baseline and after two years.

Results: Sphingomyelin (SM) concentrations, particularly, SM(d43:2), were lower in MCI Aβ+ individuals compared to controls. Further, SM(d43:2) was also nominally reduced in MCI Aβ+ individuals compared to MCI Aβ-. No plasma lipids were associated with performance on neuropsychological tests at baseline or between the two time points after correction for multiple testing.

Conclusion: Reduced plasma concentrations of SM, was associated with AD.

Introduction

Alzheimer's disease (AD) is a heterogeneous disorder where both amyloid and non-amyloid centric mechanisms could play different causative roles for the manifestations of the disease, i.e. familial vs. sporadic AD [1, 2]. The hallmarks of AD are extracellular plaques mainly containing β-amyloid peptides and intracellular neurofibrillary tangles consisting of hyper- and abnormally phosphorylated tau protein, both of which are reflected in the concentrations of the cerebrospinal fluid (CSF) biomarkers amyloid-beta42 ($A\beta_{42}$) and CSF phosphorylated tau (p-tau), respectively [1]. Despite current clinical trials focusing on altering amyloid metabolism [3], and reports of some positive results [4], no effective disease-modifying treatment is currently available [5]. It is therefore crucial to explore other disease mechanisms as potential novel treatment targets, and to that end, novel non-amyloid markers as low-cost and feasible diagnostic and prognostic blood-based biomarkers are warranted [6].

Several studies report altered blood lipid levels in sporadic AD pathology [7-10]. The human brain is a lipid-rich organ, highly abundant of cholesterol, glycerophospholipid and sphingolipid [11]. Lipids are required as energy storage and serve important structural and regulatory roles in cellular membrane formation, cellular transport, protein stabilisation and modulation, cell signalling, and regulation of gene expression [6].
The cellular membrane, exhibit lipids rafts, liquid-ordered domains rich in cholesterol, sphingolipids, including sphingomyelin (SM), and glycerophospholipids, including ganglioside 1 and 2 (GM1 and GM2, respectively) [11].
Amyloid precursor protein (APP), β- and γ-secretases are all transmembrane proteins, hence changes to the lipid raft and its composition and function might contribute to changes in β- and γ-secretase activities and consequently affect the production of $A\beta_{42}$ in AD [6, 12]. Investigating lipid homeostasis alterations during AD pathogenesis will complement the proteomic approaches channeled towards the development of early diagnosis of AD and possibly also AD progression [6]. These studies should be done in well characterized longitudinal cohorts aiming to link blood-based lipidomic changes with neuropathology and to integrate findings with known genomic and proteomic alterations in AD, and might make way for novel disease-modifying treatments [6, 9] although this has not always been the case in most previous studies.

In this study we explore baseline plasma lipid profiling aiming to identify as many lipids as possible in participants with mild cognitive impairment (MCI) with and without AD pathology. We hypothesized that specific plasma lipids would associate with MCI due to AD, and possibly predict disease progression of cognitive impairment at two-year follow-up.

Methods

Materials

Participants were drawn from the Norwegian multicentre longitudinal cohort study "Dementia Disease Initiation" (DDI) [3], which included participants from 2013 from referrals to memory clinics, or self-referrals from advertisements in media. Cognitively healthy controls were included among spouses of participants and from patients who had had a lumbar puncture for orthopedic surgery. Criteria for inclusion were age between 40 and 80, and native language from one of the three Scandinavian countries. Participants underwent a comprehensive evaluation including a full medical history, physical and neurological examinations and brain imaging in addition to blood tests and lumbar puncture. More details of recruitment and diagnostic procedures have been described previously [3]. The cognitive

examination battery included the Mini Mental State Examination (MMSE) [13], verbal learning and memory (CERAD word list test) [14], visuoperceptual ability (VOSP silhouettes) [15], psychomotor speed (Trail Making Test A: TMT-A), divided attention (TMT-B) [16] and verbal fluency (COWAT) [17]. Except for the MMSE, standardized T-scores (M=50, SD=10) were calculated for the tests based on demographically adjusted norms [15, 18, 19]. The Clinical Dementia Rating (CDR) Scale was also used to assess cognitive and daily functioning [20] based on interviews of participants and an informant by a physician alone or together with a psychologist. The cognitive assessment is performed approximately every 2 years. Research staff participated in bi-annual meetings, with case discussions to align procedures.

Out of the 658 participants available from the DDI cohort in January 2019, we identified 50 Aβ+ (as defined below) MCI participants, who were sex and age matched by manual matching with 50 Aβ- MCI and 50 healthy controls, who, if possible, had completed the first follow up visit two years after baseline. One participant was later excluded due to later being reclassified as not having MCI. There were a few (1-5) missings on cognitive tests, and 138 had complete follow-up cognitive assessment after an average of 24.5 months.

Cognitive test battery and standardized classification of MCI diagnosis
The NIA-AA criteria were used to classify MCI, requiring reporting of subjective cognitive impairment or decline, verified objectively by low performance on clinical cognitive tests in one or more cognitive domains [21, 22]. The cutoff value for MCI (defined as normal versus abnormal cognition) was results ≤ 1.5 standard deviations below the age, sex and education adjusted normative mean on either CERAD word list (delayed recall) [18], TMT-B, COWAT [19] or VOSP silhouettes (this test was only adjusted for age) [15].

CERAD memory composite score
In order to provide a robust measure of memory function, we constructed a memory composite score comprising subtests from The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) wordlist memory test (WLT). The composite included CERAD subtest total learning, recall and recognition and was constructed following an established method for cognitive composites [23, 24]. Similar CERAD memory composite scores have previously been shown accurate in detecting prodromal AD [25]. Briefly, raw scores for CERAD subtest total learning (30 items), recall (10 items) and recognition (20 items) were standardized to a score between 0 – 1. Then, these scores were summed and averaged to compute a 0 – 1 standardized composite score. In order to provide normative adjustment for pertinent demographics, a regression-based norming procedure [18, 26] was employed using n=146 healthy controls from the DDI cohort [3]. Standardized T-scores were then calculated for the participants in the present study. (See supplementary material, including supplementary table S1, S2 and S3 for a full description).

Blood and cerebrospinal fluid
Blood samples were drawn, collected and handled according to standardized procedures and then shipped to and stored at the main study center Akershus University Hospital (AHUS) and then shipped to the United Kingdom (UK) for lipid profiling (see below). Serum lipid analyses were performed locally at each center according to local procedures including total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides. At two centres LDL cholesterol measurements were not done. For lipid profiling analyses EDTA blood samples were drawn, centrifuged at 1200 g for 13 min before plasma was aliquoted in polypropylene tubes and stored at -80°C. Time from venipuncture until aliquoted plasma was frozen was below 2 hours. Plasma was kept at -80°C until analysis.

Plasma lipid profiling was performed at King's College London, UK, using methods as described in O'Gorman et al [27]. Briefly, 20uL of plasma sample was added to a 2mL Eppendorf tube. 20uL of 0.9% w/v NaCL (aq), 56uL of Chloroform/Methanol (2:1) containing 14 internal standards (10ug/mL for all) and 184uL of Chloroform/methanol (2:1) were added to the Eppendorf tube containing samples. The mixture was then vortexed and centrifuges at 1000g for 10 minutes under 4°C. The lipids containing lower chloroform layer was extracted for reverse phase analysis using ultra-high performance chromatography coupled with quadrupole time-of-flight mass spectrometer (UHPLC-QTOFMS).

The lumbar puncture procedure and CSF analyses, including $A\beta_{42}$ and total tau (t-tau) and phosphorylated tau (p-tau) analyzed at the Department of Interdisciplinary Laboratory Medicine and Medical Biochemistry at Akershus University Hospital have been described previously [3, 28]. The CSF $A\beta_{42}$ measurements were dichotomized using a cutoff of ≤ 708 ng/L previously determined in a DDI PET [18F]-Flutemetamol uptake study [29].

Ethics

All participants signed a written informed consent and the study was approved by the Regional Ethics Committee (2013/150). The entire study conduct was in line with the guidelines provided by the Helsinki declaration of 1964 (revised 2013) and the Norwegian Health and Research Act.

Statistical analyses

Demographic and serum lipids statistical analyses

SPSS version 24 was used for statistical analyses for demographical baseline data and serum lipids. Normality was assessed by inspection of QQ-plots, histograms and the Shapiro-Wilk test of normality. For continuous variables with normal distributions, between-group comparisons were carried out using one-way ANOVAs. For the continuous variables of non-normal distributions, between-group comparisons were performed with the Kruskal-Wallis tests. For statistically significant ANOVA, post-hoc Bonferroni (equal variances assumed) or Thamhane's T2 (equal variances not assumed) were applied. For Kruskal-Wallis tests Bonferroni adjusted Dunn's pairwise comparisons were performed. For dichotomous variables, between group comparisons were performed using Chi-square tests.

Plasma lipid analyses

RStudio (1.2.1335) was used for statistical analyses of the plasma lipids. All plasma lipids were normalized using inverse normal transformation (INT). Two participants with extremely high BMI were removed as outliers.

The primary analyses were the association of plasma lipids with a) diagnosis i.e MCI Aβ+, MCI Aβ- and healthy controls, and b) memory function as measured by the baseline CERAD composite T score and the change between baseline and follow-up as residualized change score (Δ) as described below (ΔCERAD composite T-score).

Secondary analyses were the associations of plasma lipids with the baseline of the other cognitive tests, i.e CERAD learning T-score, CERAD recall T-score, TMT A T-score, TMT B T-score, COWAT T-score, and VOSP T-score, and the change between baseline and follow-up (ΔCERAD learning T-score, ΔCERAD recall T-score, ΔTMT A T-score, Δ TMT B T-score, ΔCOWAT T-score, and ΔVOSP T-score).

In preliminary analyses, linear regression analyses were used in order to investigate the association of each plasma lipid with each potential covariate (age, sex, education, BMI, HDL, LDL, TG, DM, HC, HT, lipid lowering medication, smoking status and APOE). In the

main univariate analyses both logistic and linear regression analyses were performed with diagnosis and cognition as the respective outcomes. Briefly, logistic regression was used to investigate the association of lipids with diagnosis at baseline and linear regression analyses were used to investigate the association of lipids with CERAD composite T score at baseline and the ΔCERAD composite T-score. Linear regression analyses were also run using all secondary outcomes. All logistic and linear regression analyses were adjusted for BMI, HC, HT and smoking status. As a next step the analyses were adjusted for APOE status. To calculate the change (Δ) in all cognitive outcomes between baseline and follow-up, each cognitive test at follow-up was regressed against the baseline and the residuals were used (further adjusted for months of follow-up). A Bonferroni threshold of $p<0.005/70$ was used whereby 70 is the number of lipid principle components explaining >95% of variation in lipids following principal component analysis. For the binary outcomes, the Odds Ratios (OR) represent the odds ratio for being MCI Aβ+ per 1-SD (INT transformed) metabolite concentration and for the continuous outcomes the β-regression coefficients (beta) represent the change in the respective T-score between T1 and T2 per 1-SD (INT transformed) metabolite concentration.

As lipids are highly correlated and the number of variables exceed that of the observations (p>n), multivariate analysis was also performed on the main outcomes to observe whether associations between the lipids and the tested outcomes remained when taking into account lipids intercorrelation, and to identify which lipids are strong contributors to the outcomes. Two types of multivariate analyses, PLS-DA and Random Forests (RA), were run on the main outcomes. All lipids were regressed against all covariates and the lipid residuals were used for downstream multivariate analyses. Internal cross-validation was used (data was internally split into 75-25 train-test and 1000 bootstraps took place and average results presented).

Results

The baseline characteristics of the 149 participants are presented in Table 1. There were no significant differences between the groups regarding age, sex, education, medical history, smoking status, BMI or serum lipid status. The three groups differed regarding cognitive test scores (Table 1). A total of 261 lipids were identified, and annotated as ceramides (Cer) diacylglycerols (DG), phosphatidylcholines (PC), Lysophosphatidylcholines (LPC), phosphatidylethanolamines (PE), phosphatidylinositols (PI), sphingomyelins (SM) or triglycerides (TG). Most lipids were found to be inter-correlated (Supplementary Figure 1) and associated with many of the covariates (Supplementary Figure 2)

Plasma lipid profile and MCI-AD vs MCI non-AD

A sphingomyelin (SM(d43:2) was the only lipid associated with MCI Aβ+ compared to controls after passing correction for multiple testing, being decreased in MCI Aβ+ (OR=0.29, 95% CI 0.14-0.56, $p=6.2 \times 10^{-4}$) (Figure 1A). An additional 17 lipids were associated with MCI Aβ+ compared to controls at $p<0.05$. Further, 11 of these lipids were also associated with MCI Aβ+ compared to MCI Aβ- but no association passed correction for multiple testing (Figure 1B), with the strongest association at $p<0.05$ being with a TG (TG(60:2) (OR=2.32, 95% CI 1.30-4.41, $p=6.4 \times 10^{-3}$). It was also observed that SM(d43:2) was associated with MCI Aβ+ compared to MCI Aβ- at $p<0.05$ (OR=0.52, 95% CI 0.29-0.89, $p=2.1 \times 10^{-2}$) (Figure 1C).

Plasma lipid profile and cognitive impairment
No associations with CERAD composite T-score at baseline or ΔCERAD composite T-scores passed correction for multiple testing (Figure 2A&B). Altogether 8 lipids were associated with CERAD composite T-score at baseline at $p<0.05$, the strongest association being with a Phosphatidylinositol (PI), PI(36:4) (beta=-2.82, 95% CI -4.9 to -0.74, $p=8.2 \times 10^{-3}$), and; two no lipids were associated with ΔCERAD composite T-scores (the strongest association being with PC(O-36:0) (beta=-0.182, 95% CI -0.37 0.001, p=0.05).

Regarding the secondary outcomes, two associations passed correction for multiple testing. These were both with two PIs (PI(38:3) and PI(38:4)) and baseline VOSP T-score (beta=-3.98, 95% CI -6.0 – -2.00 $p=1.12 \times 10^{-4}$ and beta=-3.65, 95% CI -5.59 – -1.71, p $p=2.89 \times 10^{-4}$ respectively) (Supplementary Figure 3).

The associations of all lipids with all main and secondary outcomes are presented in Supplementary Table 4 and Supplementary Figure 4. There was modest overlap between the lipids associated with each outcome. Most of the overlap across the different outcomes was for PIs. For example, PI(36:4), was associated with MCI Aβ+ diagnosis, with CERAD composite, learning and recall, and with VOSP T score at $p<0.05$.

Multivariate data analysis highlighted that the lipids with the highest variable importance (VIP) in most models were the same lipids highlighted by univariate analysis (Supplementary Figure 5). This was most evident for the diagnosis models (MCI Aβ+ compared to controls), where the top lipids based on their VIP using PLS-DA(SM(d42:3) and SM(d43:2)) and RF (SM(d43:2)) were also the top molecules in univariate associations. The PLS-DA model predicted MCI Aβ+ with 0.624 accuracy (Sensitivity=0.623, Specificity=0.629 and AUC=0.638; the top model included 5 components) and the RF model with 0.662 accuracy (Sensitivity=0.691, Specificity=0.692 and AUC=0.662).

Discussion

The main finding in the current study, after screening 261 plasma lipids in individuals with MCI with and without AD pathology and healthy controls, was that a number of plasma SM concentrations, and particularly, SM(d43:2), were lower in MCI Aβ+ individuals compared to controls. Further, SM(d43:2) was also nominally reduced in MCI Aβ+ individuals compared to MCI Aβ-. Although no lipids were associated with CERAD composite T-score at baseline, or with ΔCERAD composite T-score after correction for multiple testing, a number of PI showed modest negative associations with CERAD composite T-score at baseline, i.e. an increase in PI was associated with a lower baseline CERAD composite T-score. Further, a PC showed borderline negative associations with ΔCERAD composite T-score, i.e. decrease in PC is associated with an increase in CERAD composite T-score between the two time points. Regarding the secondary outcomes, two PI were found to be negatively associated with VOSP T score at baseline, i.e. increase in PI was associated with decrease in VOSP at baseline after correction for multiple testing.

Previous metabolomics studies have shown alterations in SM pathways in AD, although results are not always in agreement. A small cross-sectional study reported lower levels of plasma SM in AD patients compared to controls [30] while Toledo et al. found serum SM to be increased in AD and to be associated with worse cognitive outcomes [31]. Interlaboratory variability and methodology have been observed regarding the use of serum versus plasma and also marked differences in how they are processed. This might affect the results, further underlining the importance of consistency across laboratories [6].

Conflicting results could be due to the stage of the disease [30], as it has been reported that low levels of serum SM vary according to the timing of the onset of memory impairment, a deficit observed early in AD pathogenesis [32]. Similar results have been found concerning CSF, as Kosicek et al. reports significantly increased SM levels in CSF from individuals with prodromal AD compared to normal controls, however no change between mild and moderate AD groups and normal controls [33]. Interestingly, SM(d43:2) was detected as one of the key lipids that was altered in a study investigating the cerebrospinal fluid (CSF) lipidomic signature of ALS patients by mass spectrometry, similar to our approach, suggesting an involvement of the glyosphingolipid pathway in neurodegeneration in both AD and ALS [34].

Glycosphingolipids have been shown to bind specifically to Aβ oligomers on synaptic membranes of neurons [35]. In accordance with these data and our data on altered sphingomyelins in MCI/AD, Molander-Melin et al. showed that biochemical equivalents of lipid rafts also termed detergent resistant microdomains from frontal cortex of AD brains displayed higher concentration of ganglioside GM1 and GM2 [36] compared to normal control brains.

PI have been found to be present in tau aggregates [37], however, despite previous recommendation of linking longitudinal changes in lipids not only to Aβ levels, but also tau pathology [6], associations of PI or any plasma lipid with tau pathology was not the scope of this study. Whilst Mapstone et al. [7] found a PI to be one in ten serum lipids that can accurately predict memory loss in up to 90% of cases 2 years before the onset of dementia [7], these results could not be replicated in a later study [38]. Thus, the role of PI in cognitive impairment remains unclear.

In the current study we present norms for a CERAD composite measure. The use of a CERAD composite measure as a primary outcome could be a limitation, as this could mask domain specific cognitive functions such as learning, recall and recognition, which are qualitatively different aspects of learning and memory. However, a composite measure may also offer a more robust and reliable index of learning and memory function. A composite score capitalizes on regression towards the mean. I.e., the participant is less likely to obtain two or more low scores on several measures of learning and memory function and may be more robust against chance low performance on one measure not related to neurodegeneration or cerebral dysfunction (e.g. low motivation or inattention during a particular test). We also investigated associations with lipids and two of the subdomain measures of the CERAD word list test (learning and delayed recall) as well as other cognitive domains (psychomotor speed, executive functions, verbal fluency/language, and visual cognition) in the secondary analyses. We did not find any significant associations between lipids and the composite score or specific subdomain measures of verbal learning and memory. The utility of this measure needs to be further explored with regard to sensitivity and specificity for AD in longitudinal follow-up cohorts.

Other limitations of this study include the rather small size of the samples and potentially the non-fasting design [39]. We have done plasma lipid analyses, which have also been done by other researchers [7, 8], while others have used serum [32]. Future interlaboratory agreement regarding methodology is of importance in order to be able to replicate the findings.

The strengths of this study include the randomized and longitudinal design and the age and sex matched samples. In addition this study holds information regarding proteomics and genetics in relation to lipidomics, which has previously been identified as a potential focus for further studies [6].

Furthermore, we used logistic and linear regression analyses controlling for a number of variables that are associated with lipids and which are also known to be associated with AD and cognitive decline. These included age, sex, BMI, lipid lowering medication, smoking status, and history of hypercholesterolemia and hypertension. As the number of lipid variables is high and many lipids are inter-correlated we also employed a machine learning approach using PLS-DA and RF for the main outcomes. Results from the two approaches were in agreement, especially for the diagnosis that showed the strongest associations with lipids.

In conclusion, we found that plasma sphingomyelins concentrations, and particularly, SM(d43:2), were lower in MCI Aβ+ individuals compared to controls and also nominally reduced in MCI Aβ+ individuals compared to MCI Aβ-.
Future randomized studies with a longitudinal design, possibly with longer observational times are warranted in order to achieve additional knowledge and understanding of the lipid contribution to AD pathology.
Lipid alterations associated with AD pathology could possibly complement the proteomic approach channeled toward development of a low-cost and safe method to identify early AD pathology, progression and potential novel treatment modalities.

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Table 1. Baseline demographic and clinical characteristics							
Variable	**Groups**			*F* / χ^2, *(p)*	**ANOVA post-hoc (Bonferroni/Tamhane's)/Dunn's pairwise comparisons (*p*)**		
	1. Healthy controls n=50	2. MCI Aβ42+ n=50	3. MCI Aβ42- n=49		1 vs 2	1 vs 3	2 vs 3
Age Mean (SD)	65.0 (6.8)	65.3 (8.0)	64.9 (9.5)	*F*=0.03#, (p=0.97)			
Female n (%)	25 (50)	25 (50)	24 (49)	χ^2=0.01, (p=1.0)			
Years of education Mean (SD)	13.7 (3.0)	13.9 (3.4)	13.4 (3.3)	*F*=0.28, (p=0.75)			
Smoking count (%)							
Current smoker	5 (20)	8 (32)	12 (48)	χ^2=6.0 (p=0.2)			
Previous smoker	18 (30)	22 (37)	20 (33)				
Non-smoker	27 (43)	19 (30)	17 (27)				
BMI mean (SD)	26.3 (3.6)	25.1 (3.8)	26.6 (4.7)	*F*=1.83, (p=0.17)			
Medical history, count (%)							
Hypercholesterolemia	16 (32)	19 (38)	18 (37)	χ^2=0.4, (p=0.8)			
Hypertension	19 (38)	12 (24)	19 (39)	χ^2=3.1, (p=0.21)			
Diabetes Mellitus	3 (6)	3 (6)	2 (4)	χ^2=0.2, (p=0.89)			
Serum Lipids mean (SD)							
Total Cholesterol	5.3 (1.2)	5.7 (1.4)	5.3 (1.1)	*F=1.67*, (p=0.19)			
HDL Cholesterol	1.5 (0.4)	1.6 (0.4) n=49	1.5 (0.4)	*F=0.02*, (p=0.99)			
LDL Cholesterol	3.4 (1.2) n=47	3.9 (1.6) n=24	3.1 (0.9) n=30	*F=2.4#*, (p=0.05)			
Triglycerides median (IQR)	1.2 (0.82)	1.2 (0.82) n=49	1.1 (0.77)	χ^2*=0.90*, (p=0.64)			
CERAD composite T-score Mean (SD)	52.2 (9.6)	32.3 (9.4) n=48	40.1 (9.1) n=46	*F=57.7*, **(p<0.001)**	**p<0.001**	**p<0.001**	**p<0.001**
CERAD Learning T-score Mean (SD)	51.5 (10.2)	34.8 (8.4)	40.8 (10.4)	*F=37.9*, **(p<0.001)**	**p<0.001**	**p<0.001**	**p=0.01**
CERAD Recall T-score Mean (SD)	52.3 (9.7)	31.4 (9.6)	38.9 (10.2)	*F=57.7*, **(p<0.001)**	**p<0.001**	**p=<0.001**	**p=0.001**
TMT-A T-score Mean (SD)	47.3 (9.6) n=49	41.0 (10.6)	42.1 (9.4)	*F*=5.6, **(p=0.004)**	**p=0.006**	**p=0.032**	**p=1.0**
TMT-B T-score Mean (SD)	49.0 (7.4) n=49	37.2 (13.3)	43.1 (11.1)	*F*=16.4#, **(p<0.001)**	**p<0.001**	**p=0.007**	p=0.06
COWAT T-score Mean (SD)	49.8 (8.6) n=49	46.2 (9.7)	43.7 (7.2) n=48	*F*=6.1, **(p=0.003)**	**p=0.12**	**p=0.002**	**p=0.47**
VOSP T-score Mean (SD)	2.9 (10.4) n=49	44.0 (11.1) n=47	45.8 (11.7) n=48	*F*=8.7, **(p<0.001)**	**p<0.001**	**p=0.006**	**p=1**
CSF Aβ1-42 Mean (SD)	1010 (248)	546 (100)	1046 (196)	*F*=172.5#, **(p<0.001)**	**p<0.001**	p=0.81	**p<0.001**
CSF t-tau Mean (SD)	334 (165)	577 (305)	373 (258)	*F*=12.3#, **(p<0.001)**	**p<0.001**	p=0.75	**p=0.002**
CSF p-tau Mean (SD)	56 (20)	82 (38)	58 (32)	*F*=9.2#, **(p<0.001)**	**p<0.001**	p=1.0	**p=0.004**
***APOE*-ε4 count (%)**							
Heterozygote	22 (44)	23 (46)	19 (40)	χ^2=27.5, **(p<0.001)**			
Homozygote	1 (2)	14 (28)	1 (2)				
Non-carriers	27 (64)	13 (26)	28 (58)				

***n=sample size; F=F-statistic;* # =Welsh correction; χ^2= chi-square or Kruskal-Wallis statistic; p=p-value.**

Figure 1A

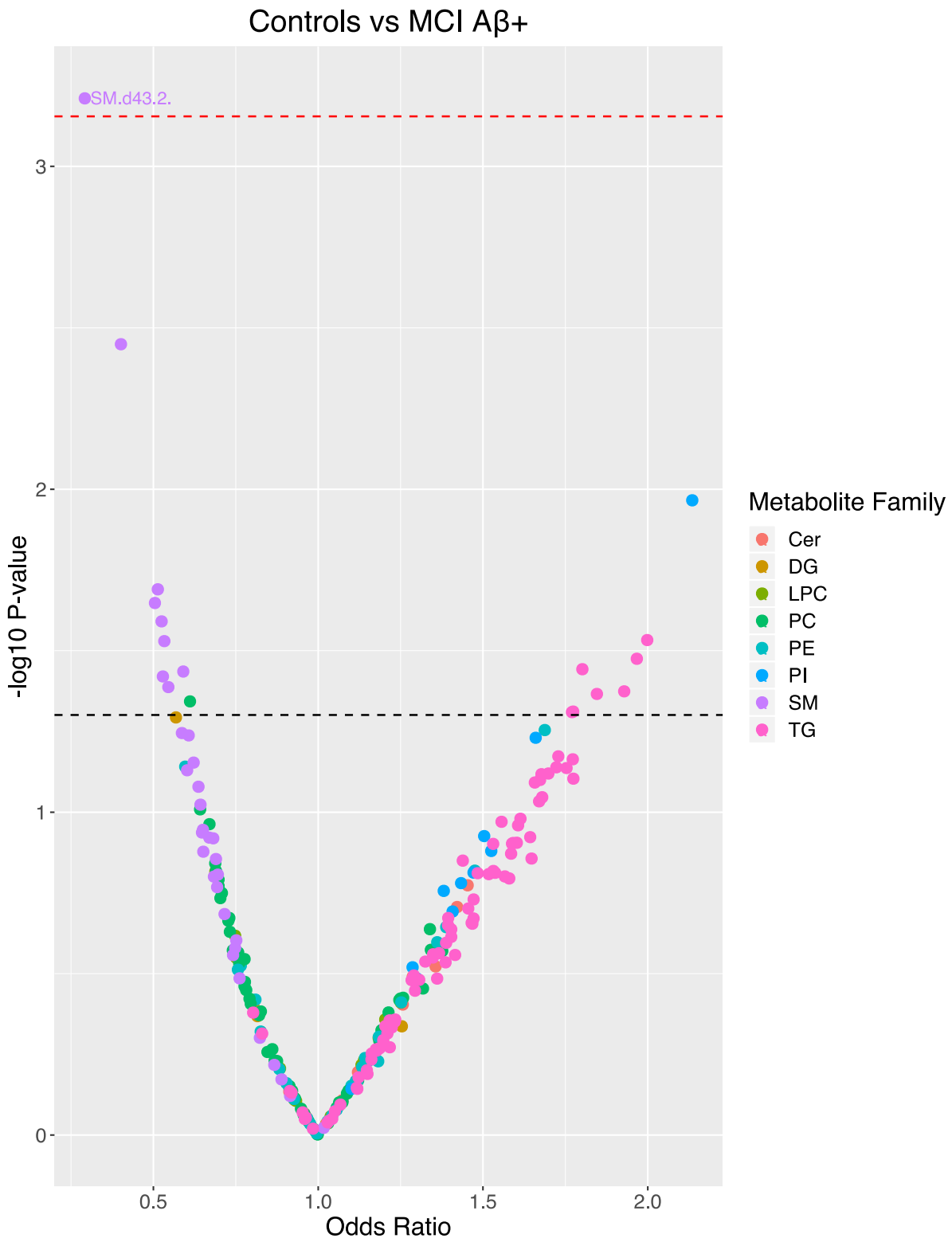

Controls vs MCI Aβ+
SM.d43.2.
3
2
1
0
-log10 P-value
0.5
1.0
1.5
2.0
Odds Ratio
Metabolite Family
Cer
DG
LPC
PC
PE
PI
SM
TG

Figure 1B

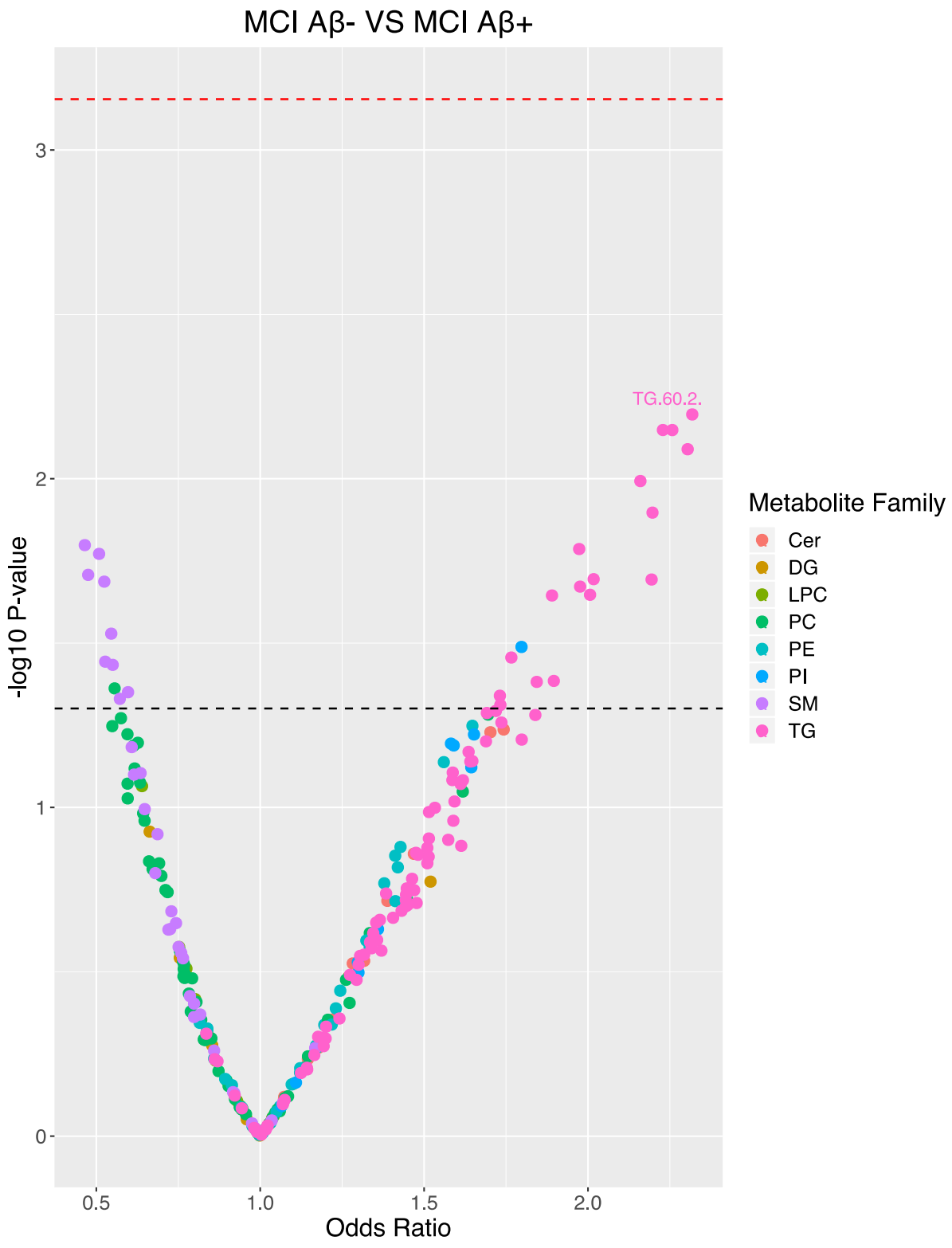

MCI Aβ- VS MCI Aβ+
-log10 P-value
Odds Ratio
0
1
2
3
0.5
1.0
1.5
2.0
TG.60.2.
Metabolite Family
Cer
DG
LPC
PC
PE
PI
SM
TG

Figure 1C

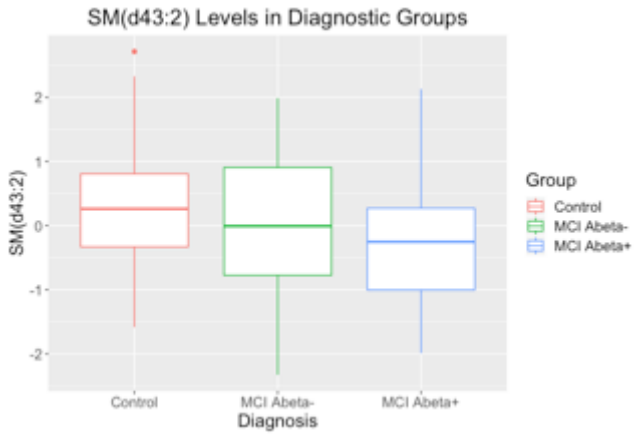


Figure 1 Legends

1. Volcano plots depicting the association of the 261 lipids with the main diagnostic outcomes: Controls vs MCI Aβ+ **(A)** and MCI Aβ- vs MCI Aβ+ **(B)** following logistic regression analyses. The black lines in the volcano plots represent the $p<0.05$ threshold and the red lines represent the multiple correction threshold at $p<0.0007$. X-axis represents the OR (MCI Aβ+ vs Controls and MCI Aβ- respectively) and y-axis represents -log10 transformed p-value from the logistic regression. **C)** Boxplot of SM.d43.2. levels (Inverse-variance transformed) in the three groups (Controls, MCI Aβ- and MCI Aβ+).

Figure 2A

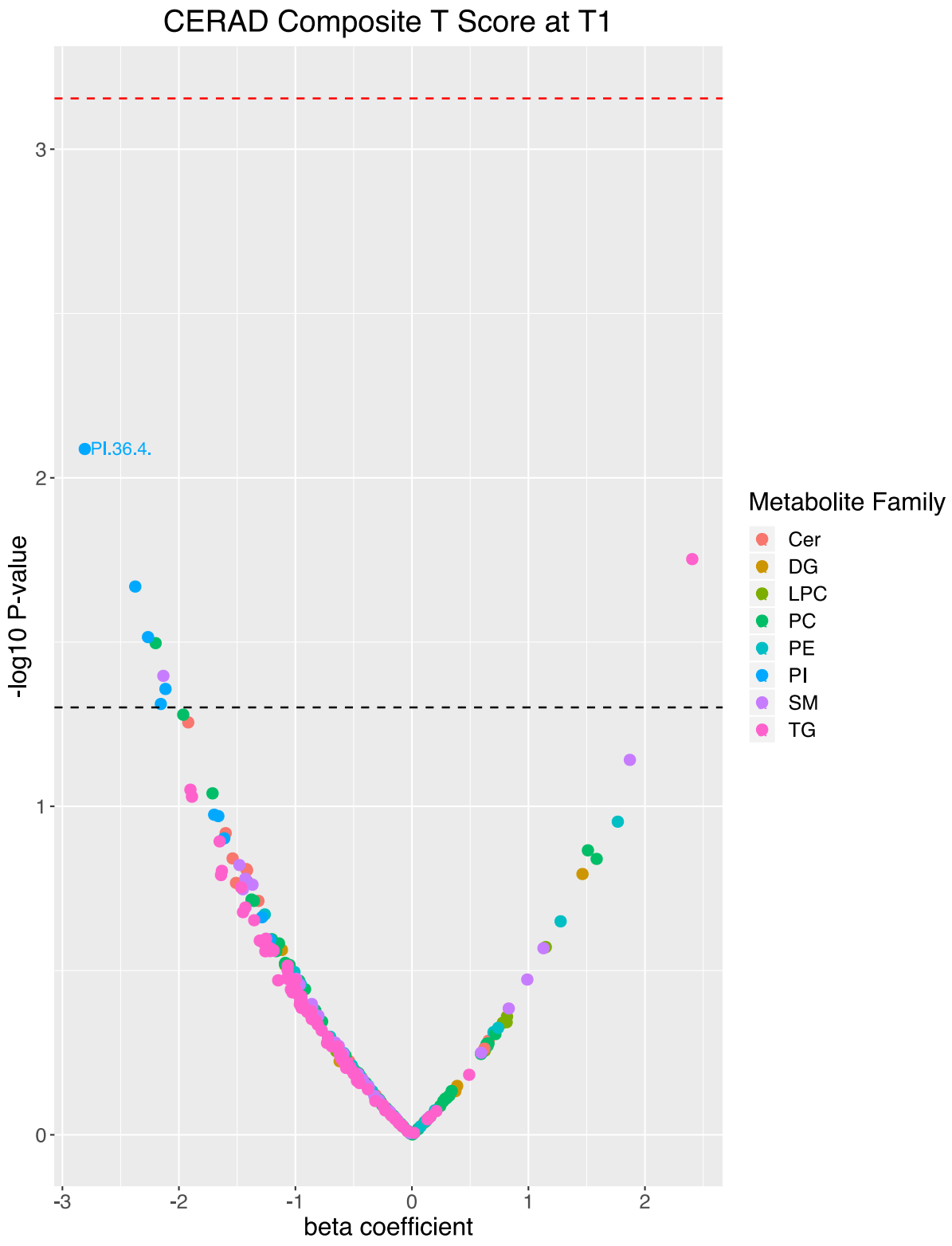

CERAD Composite T Score at T1
-log10 P-value
beta coefficient
PI.36.4.
Metabolite Family
Cer
DG
LPC
PC
PE
PI
SM
TG

Figure 2B

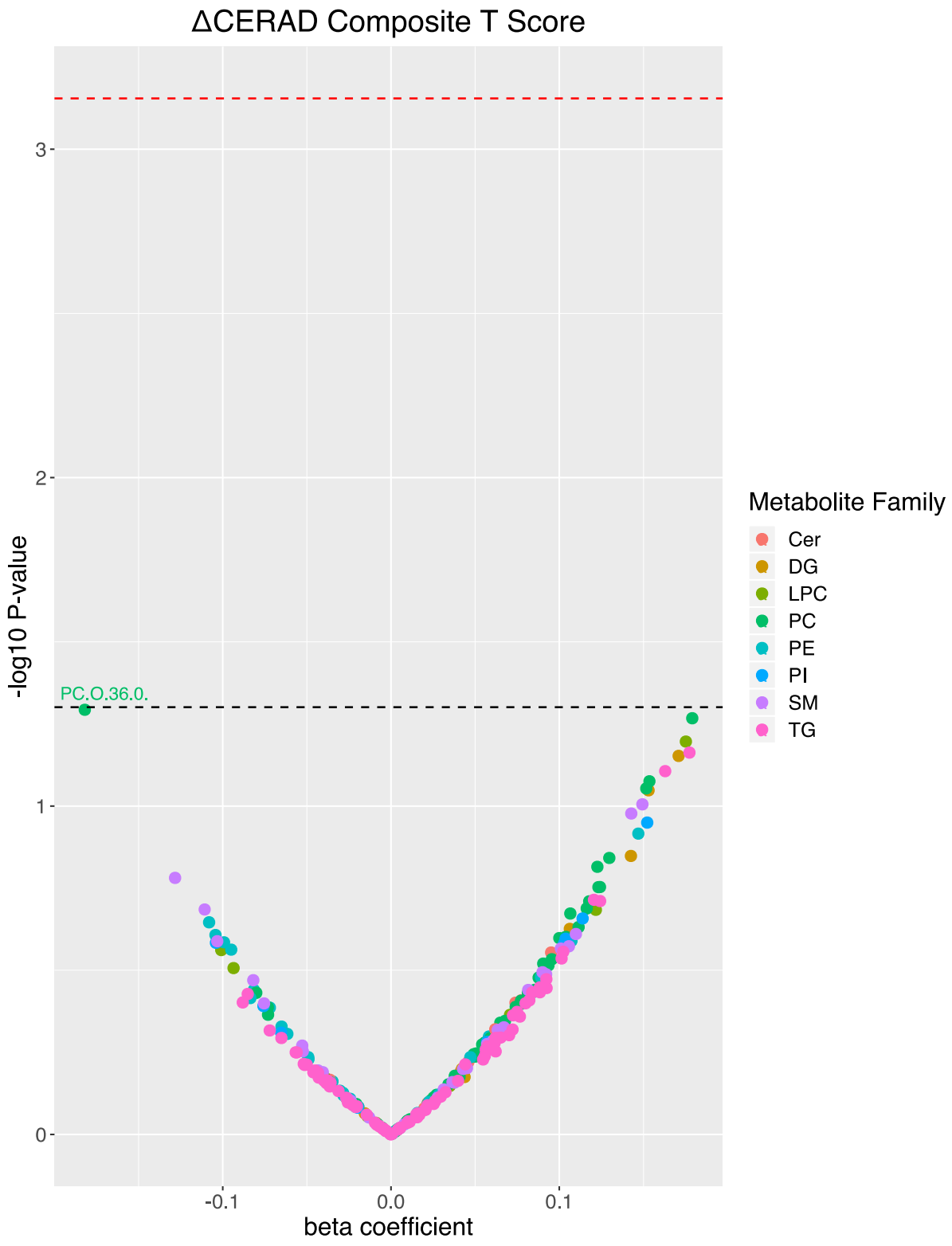

ΔCERAD Composite T Score
-log10 P-value
3
2
1
0
PC.O.36.0.
-0.1
0.0
0.1
beta coefficient
Metabolite Family
Cer
DG
LPC
PC
PE
PI
SM
TG

Figure 2 Legends

2. Volcano plot depicting the association of the 261 lipids with CERAD composite T score at T1 **(A)** and ΔCERAD composite T score **(B)** following linear regression analyses. The black lines in the volcano plots represent the $p<0.05$ threshold and the red lines represent the multiple correction threshold at $p<0.0007$. X-axis represents the beta coefficient (change in T score per 1-SD increase in Inverse-variance transformed lipid levels) and y-axis represents -log10 transformed p-value.

Supplementary material

Demographically adjusted CERAD memory composite age 41-80 years

Procedure for construction of the CERAD memory composite

Raw scores for CERAD subtest total learning (30), recall (10) and recognition (20) was standardized to a score between 0 – 1 using the following the formula: *(raw score - minimum possible score)/(maximum possible score - minimum possible score).* Then, these scores were summed and averaged to compute a 0 – 1 standardized composite score.

In order to provide normative adjustment for pertinent demographics, a regression-based norming procedure was employed.

Normative adjustment of the CERAD memory composite

Norms were based on the performance of n=146 healthy normal controls from the DDI study[3]. We used regression norming procedures similar to Kirsebom et al [18] and Testa el al [26] which requires 5-6 times smaller sample size compared to conventional discrete norming procedures [40]. Demographics of the normative sample are shown in Table 1. We first normalized the control group standardized composite score by retrieving the cumulative frequency distribution for the score. The resulting distribution was converted into a standard scaled score with a mean of 10, and a standard deviation of 3 (Table 2). We then regressed the resulting scaled scores on age, gender and education. Plots of standardized residuals predicted values were assessed to ensure that the assumption of homoscedasticity was not violated, and normality of the residuals was checked visually with Q-Q plots.

Demographically adjusted T-scores are computed using the following stepwise procedure: 1) Look up the scaled score for a given subtest in Table 2. 2) Use the regression coefficients found in Table 3 to obtain a predicted scaled score ***[intercept + individual age(coefficient for age) + individual gender(coefficient for gender) + individual years of education(coefficient***

for education)]. 3) Then, subtract the actual scaled score from the predicted scaled score and divide it by the standard deviation of the residual (Table 3) to obtain a standardized z score which may be converted to a T score [T = z(10)+50].

Table S1. Healthy control group demographics

Variable	DDI Healthy controls n=146
Age Mean (SD)	62 (8.9)
[Range]	[41-80)
Years of education Mean (SD)	14.3 (3.2)
[Range]	[8-23]
Female n (%)	85 (58 %)

Table S2. Unadjusted CERAD composite to scaled score conversion

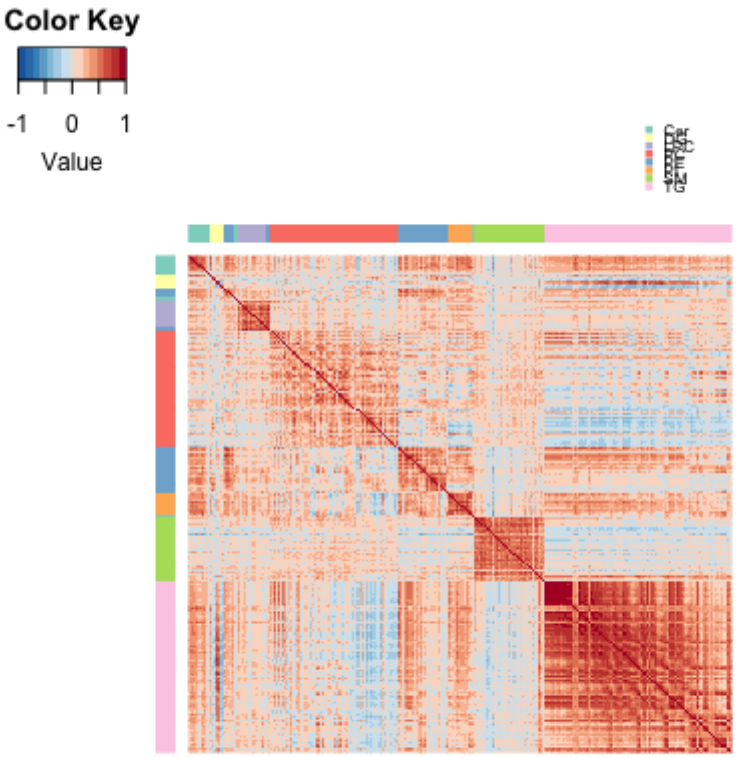
Scaled score	Standardized average of CERAD composite
3	≤0.4278
4	.4279-.4330
5	.4331-.5908
6	.5909-.6278
7	.6279-.6889
8	.6890-.7444
9	.7445-.7889
10	.7890-.8361
11	.8362-.8645
12	.8646-.8889
13	.8890-.9222
14	.9223-.9530
15	.9531-.9556
16	.9557-.9784
17	.9785-.9889
18	≥9890

Table S3. Normative regression models for the CERAD memory composite score

Variable	Predictor	B	Standard error B	T	P	Partial R^2	SD Residual
CERAD Learning							2.47311
	Intercept	15.269	1.874	8.146	<.0001		
	Age	-0.129	0.024	-5.420	<.0001	0.17	
	Education	0.170	0.064	2.671	<.01	0.05	
	Sex	1.210	0.423	2.857	<.001	0.05	

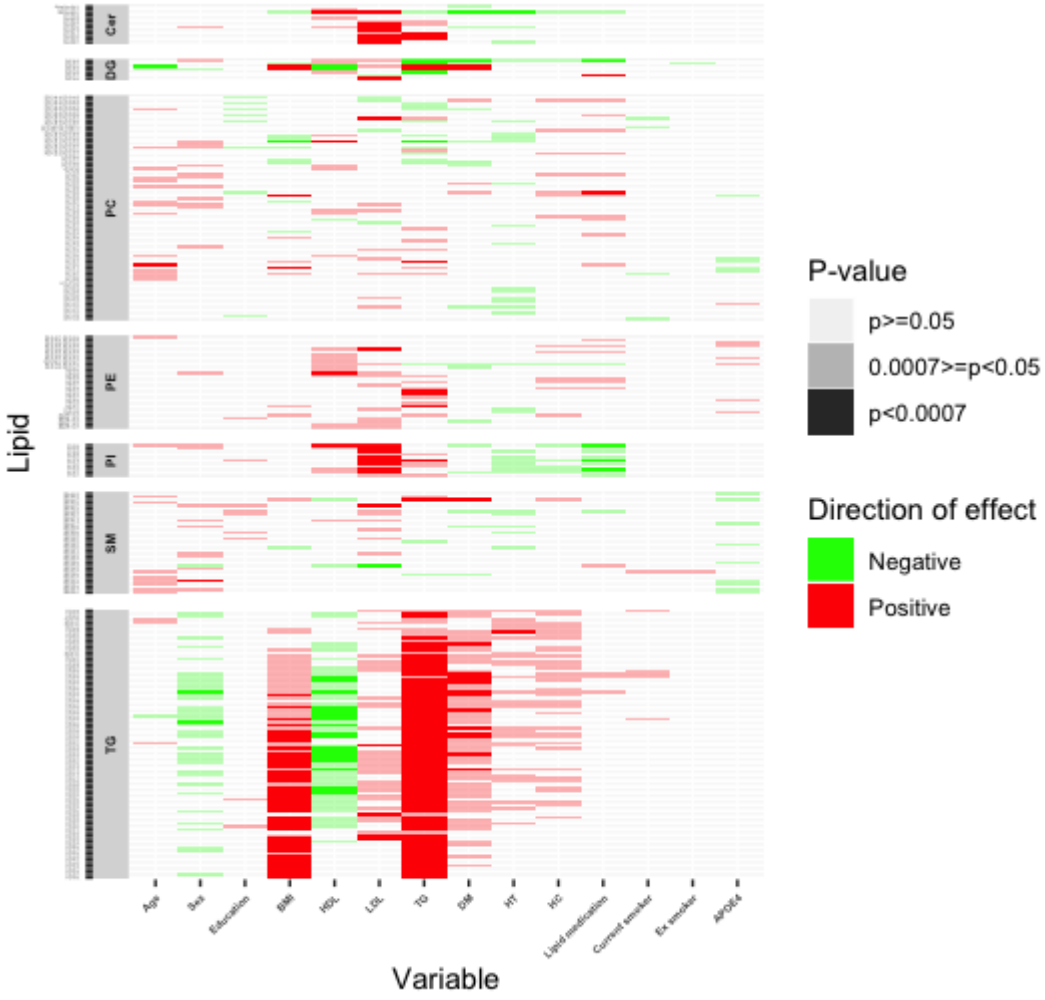
Notes. B = unstandardized regression coefficient; T = the t test statistic; SD = standard deviation.

Supplementary Figure 1



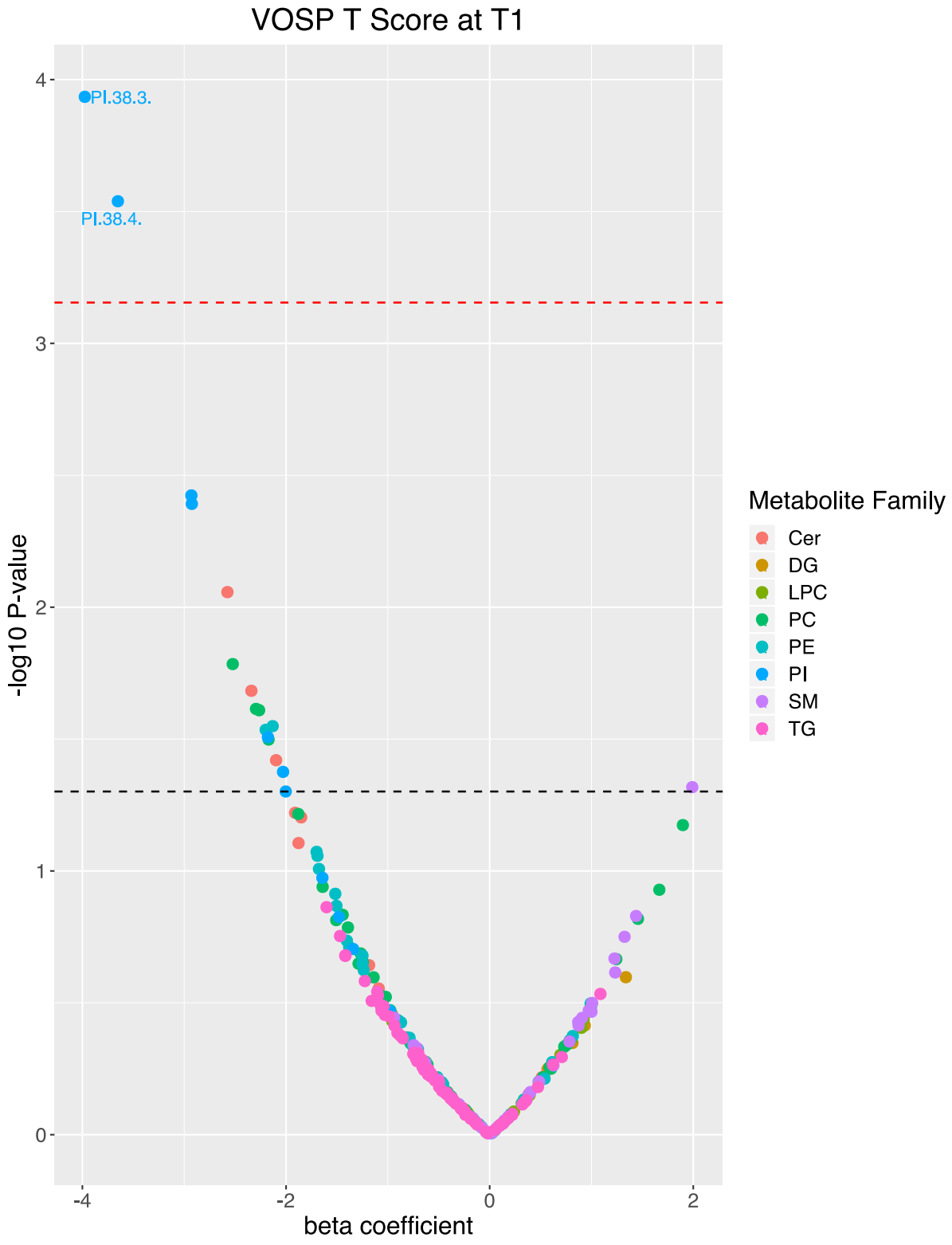
Supplementary Figure 1. Heatmap depicting pairwise correlations of lipid measures. Colours represent the Pearson's correlation coefficients (rho) with positive correlations in red and, negative correlations in blue. Lipids are ordered by lipid family.

Supplementary Figure 2



Supplementary Figure 2. Heatmap depicting the associations between each the 261 lipids and each of the covariates. Color denotes the direction of effect between each lipid and each covariate after regressing each lipid against each covariate (red: positive association, green negative association). Color intensity represents p-value with associations with p>=0.05 being in white. The Bonferroni corrected p-value threshold is p<0.0007.

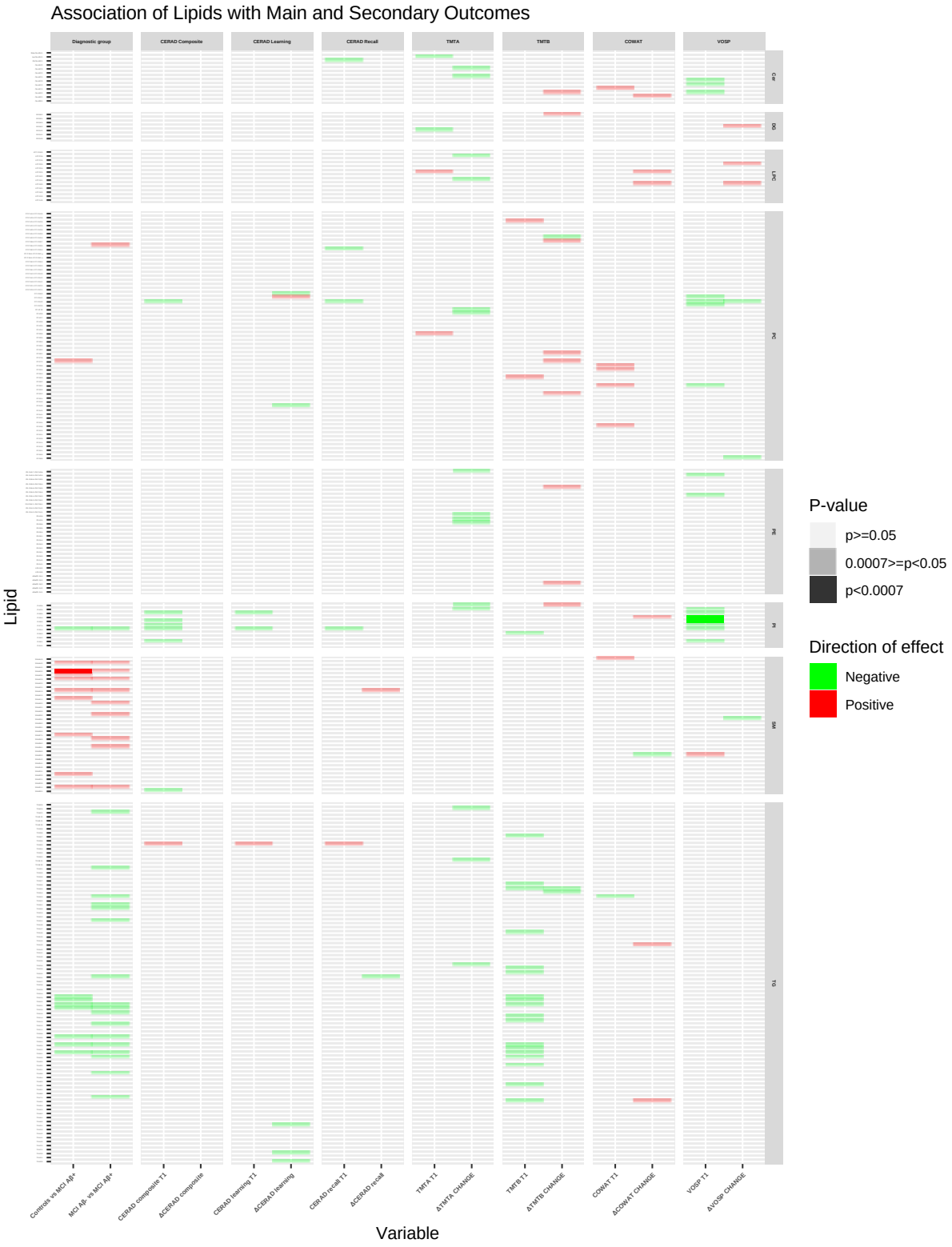
Supplementary Figure 3.



Supplementary Figure 3. Volcano plot depicting the association of the 261 lipids with VOSP T score at T1, following linear regression analyses. The black lines in the volcano plots represent the p<0.05 threshold and the red lines represent the multiple correction threshold at

$p<0.0007$. X-axis represents the beta coefficient (change in T score per 1-SD increase in Inverse-variance transformed lipid levels) and y-axis represents -log10 transformed p-value.

Supplementary Figure 4.

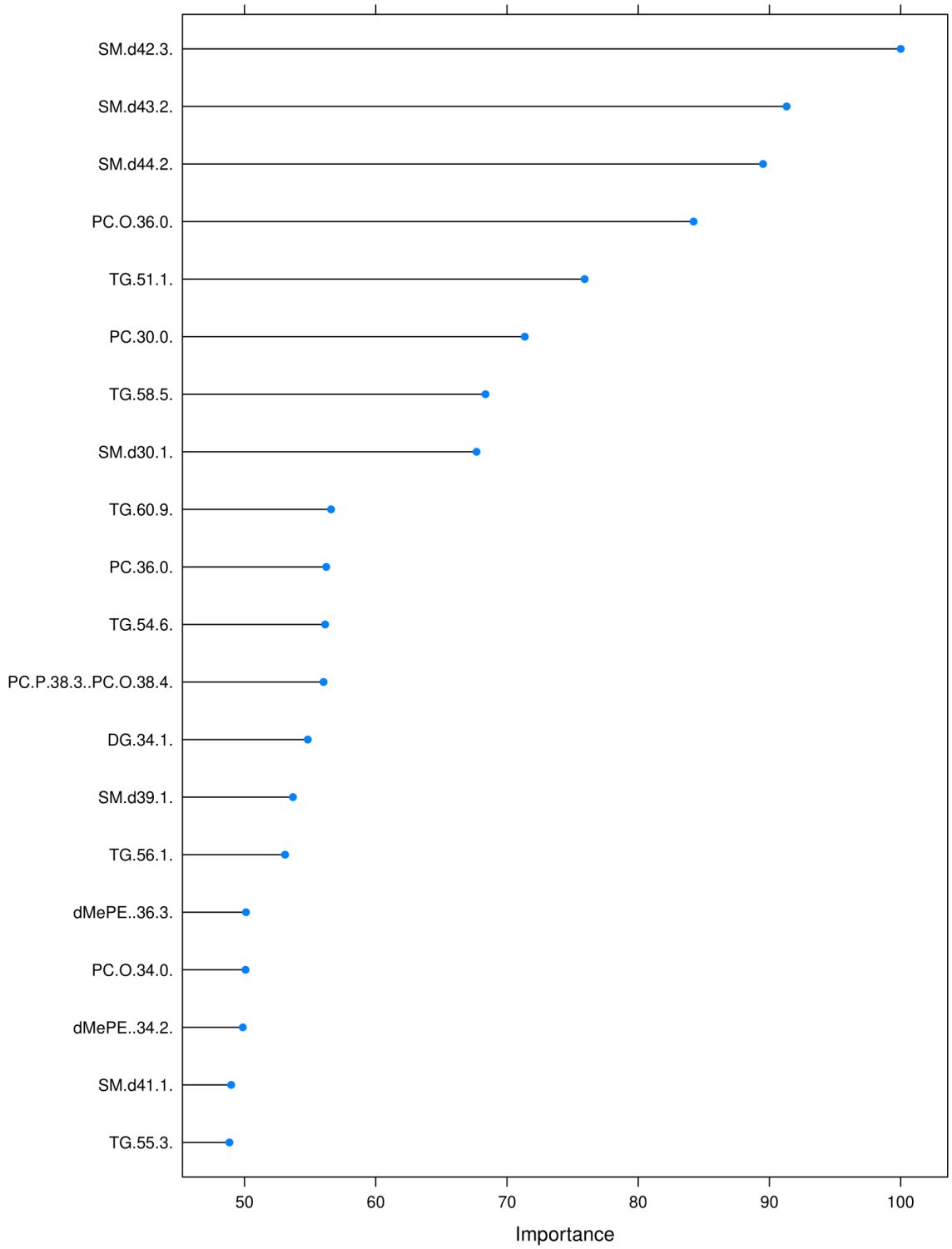


Legend Figure 4. Heatmap depicting the associations between each the 261 lipids and each outcome (Controls vs MCI Aβ+, MCI Aβ- vs MCI Aβ, and all the cognitive outcomes at T1 and the residualised change (Δ) between T1 and T2), following logistic and linear regression

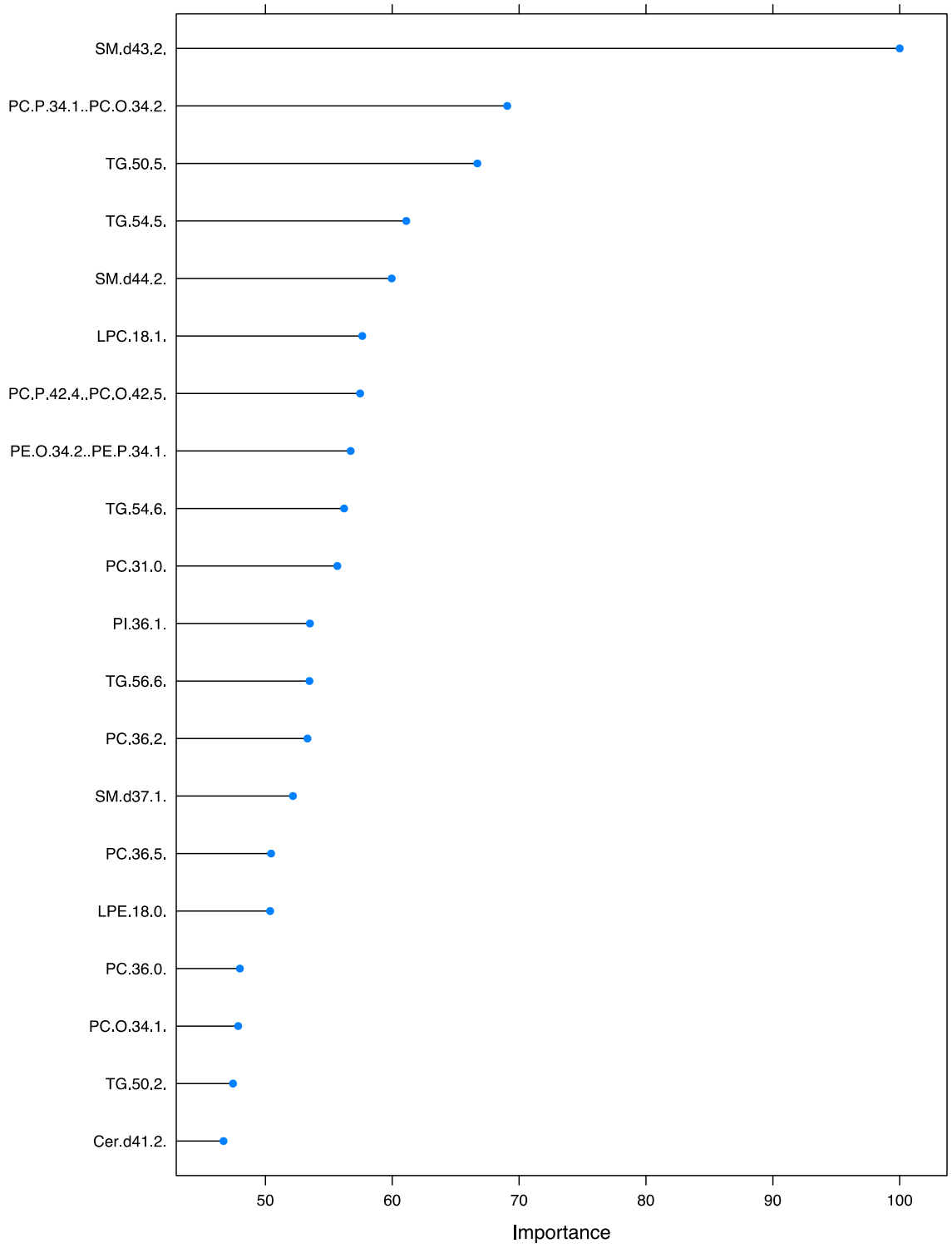
analyses. For cognitive outcomes color denotes the direction of effect (beta) between each lipid and each outcome after linear regression analyses (red: positive association, green negative association). For the diagnostic outcomes, red indicates increased odds in controls (i.e. a positive diagnostic outcome) and green indicates decreased odds in controls, in order to be able to directly compare with the continuous cognitive outcomes. Color intensity represents p-value; associations with $p>=0.05$ are in white. The Bonferroni corrected p-value threshold is $p<0.0007$.

Supplementary Figure 5.

5A



5B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TG.50.5.
TG.54.5.
SM.d44.2.
LPC.18.1.
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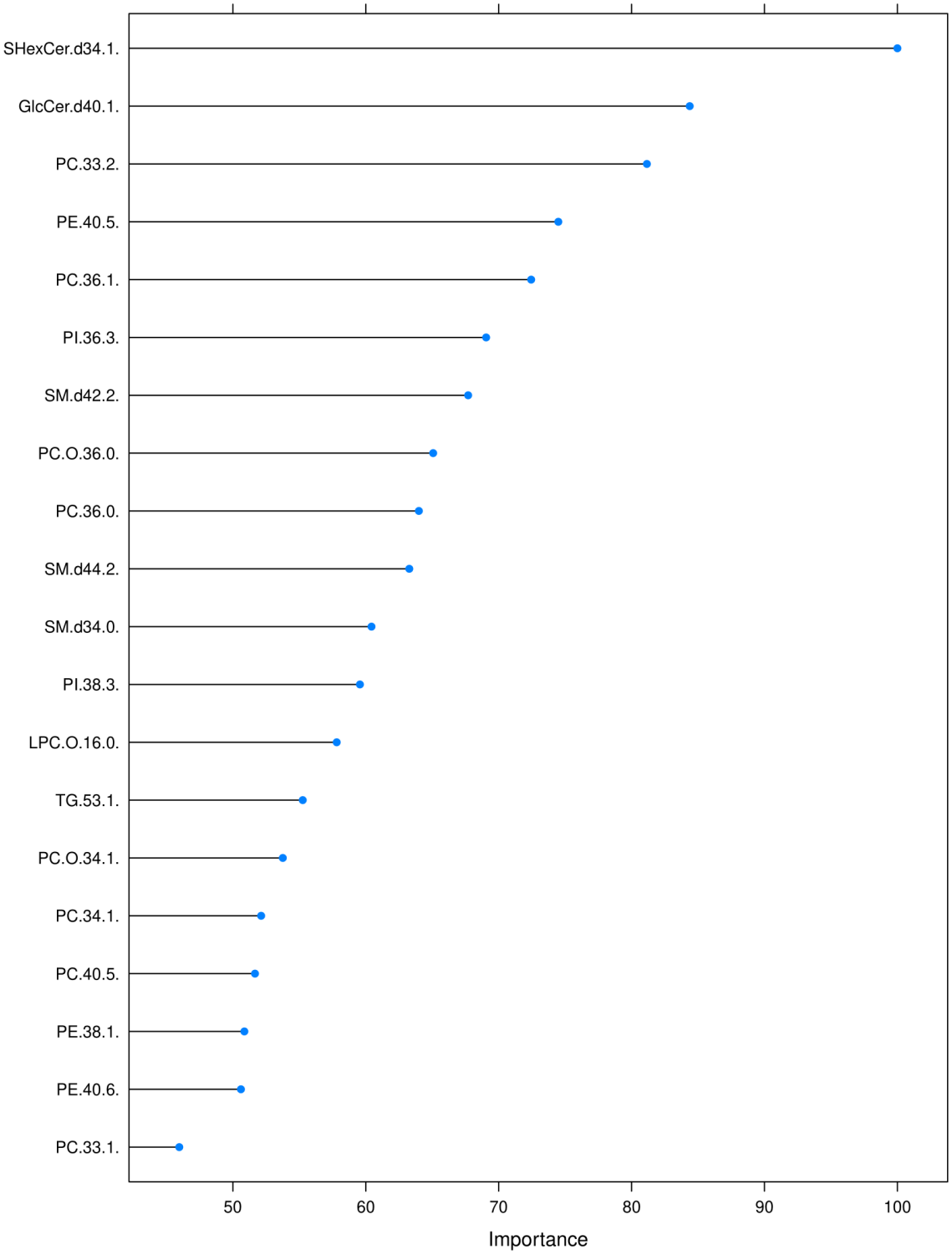
PE.O.34.2..PE.P.34.1.
TG.54.6.
PC.31.0.
PI.36.1.
TG.56.6.
PC.36.2.
SM.d37.1.
PC.36.5.
LPE.18.0.
PC.36.0.
PC.O.34.1.
TG.50.2.
Cer.d41.2.
50
60
70
80
90
100
Importance

5C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GlcCer.d40.1.
PC.33.2.
PE.40.5.
PC.36.1.
PI.36.3.
SM.d42.2.
PC.O.36.0.
PC.36.0.
SM.d44.2.
SM.d34.0.
PI.38.3.
LPC.O.16.0.
TG.53.1.
PC.O.34.1.
PC.34.1.
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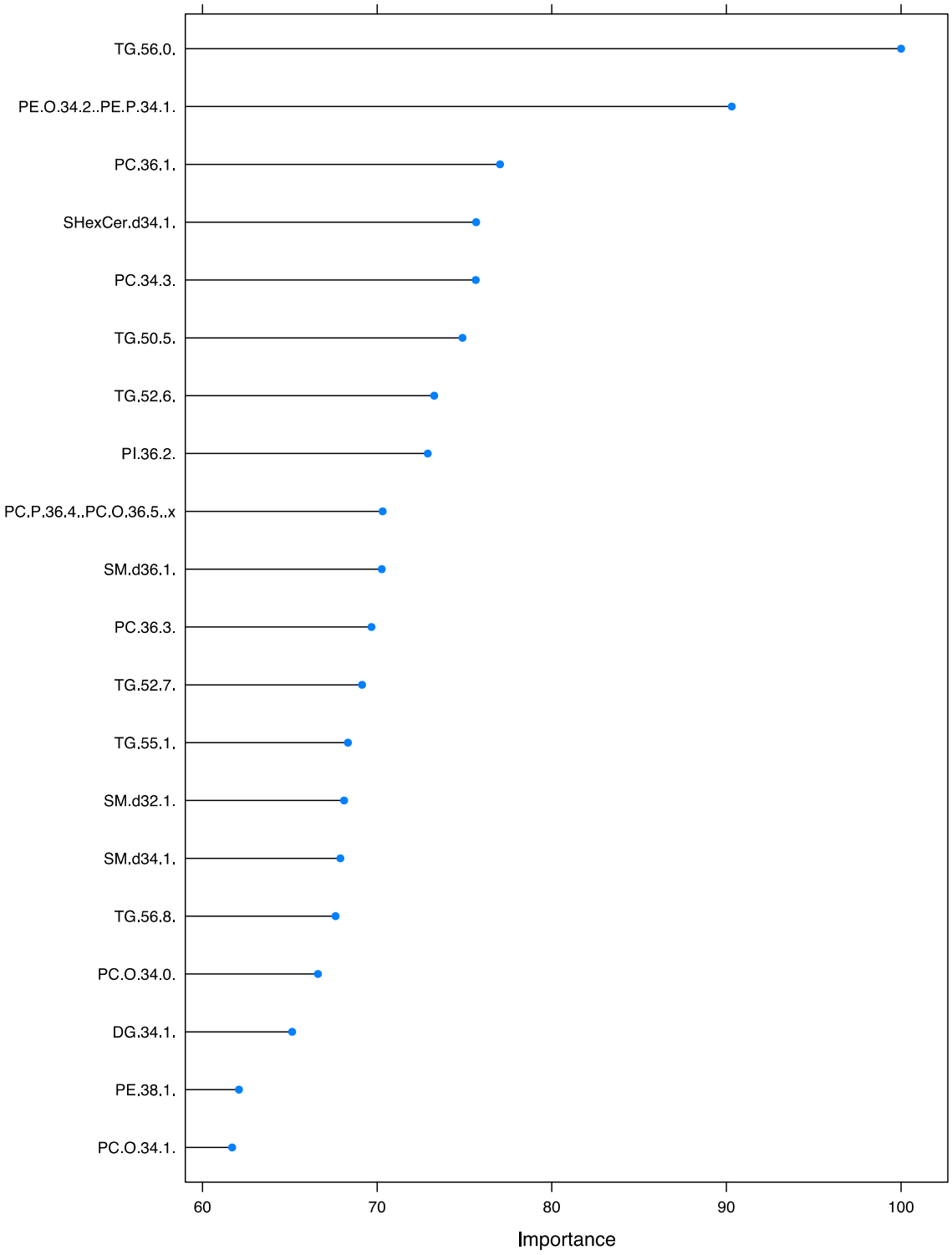
50
60
70
80
90
100
Importance

5D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PC.34.3.
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TG.52.6.
PI.36.2.
PC.P.36.4..PC.O.36.5..x
SM.d36.1.
PC.36.3.
TG.52.7.
TG.55.1.
SM.d32.1.
SM.d34.1.
TG.56.8.
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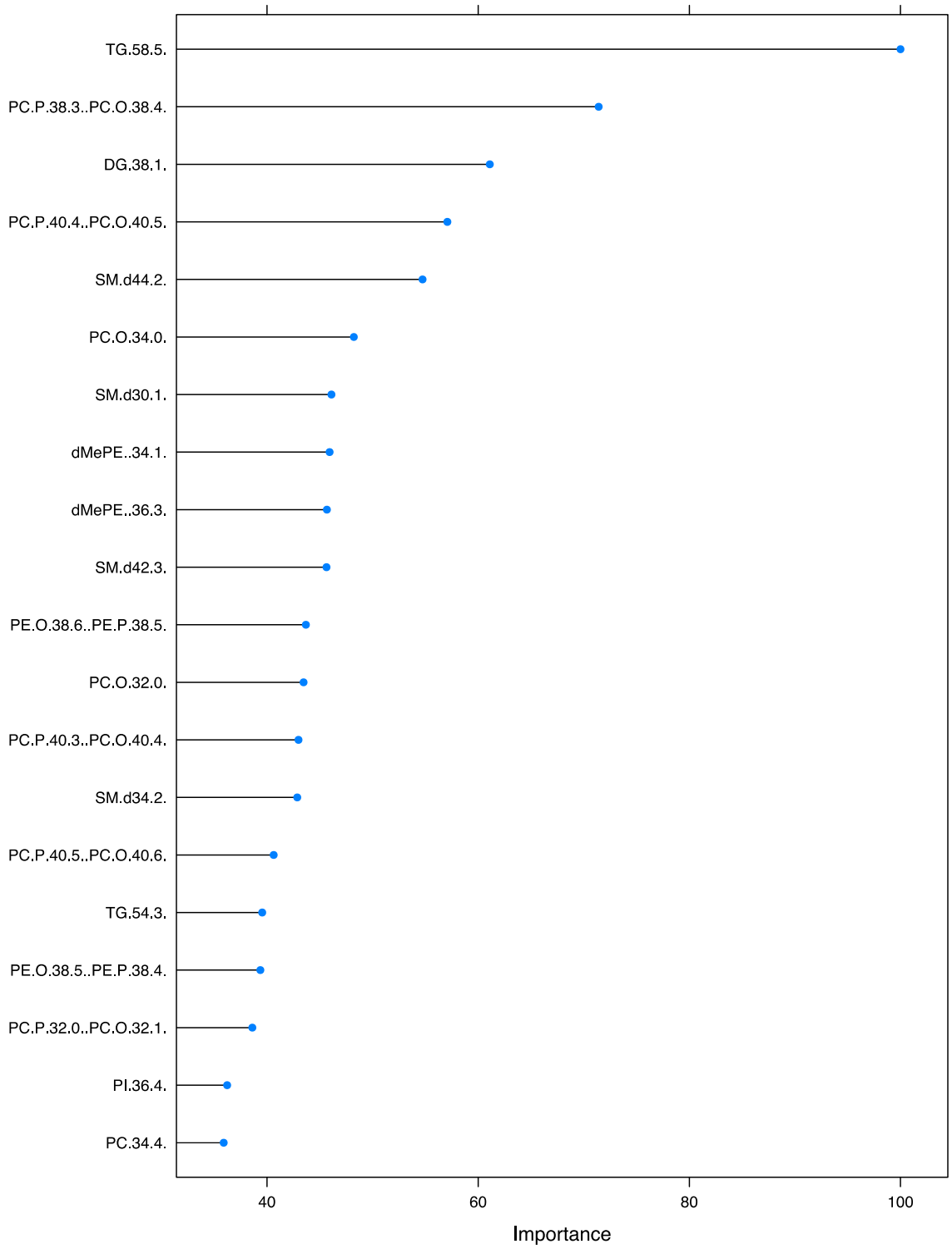
DG.34.1.
PE.38.1.
PC.O.34.1.
60
70
80
90
100
Importance

5E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PC.O.34.0.
SM.d30.1.
dMePE..34.1.
dMePE..36.3.
SM.d42.3.
PE.O.38.6..PE.P.38.5.
PC.O.32.0.
PC.P.40.3..PC.O.40.4.
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PC.P.40.5..PC.O.40.6.
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PE.O.38.5..PE.P.38.4.
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PI.36.4.
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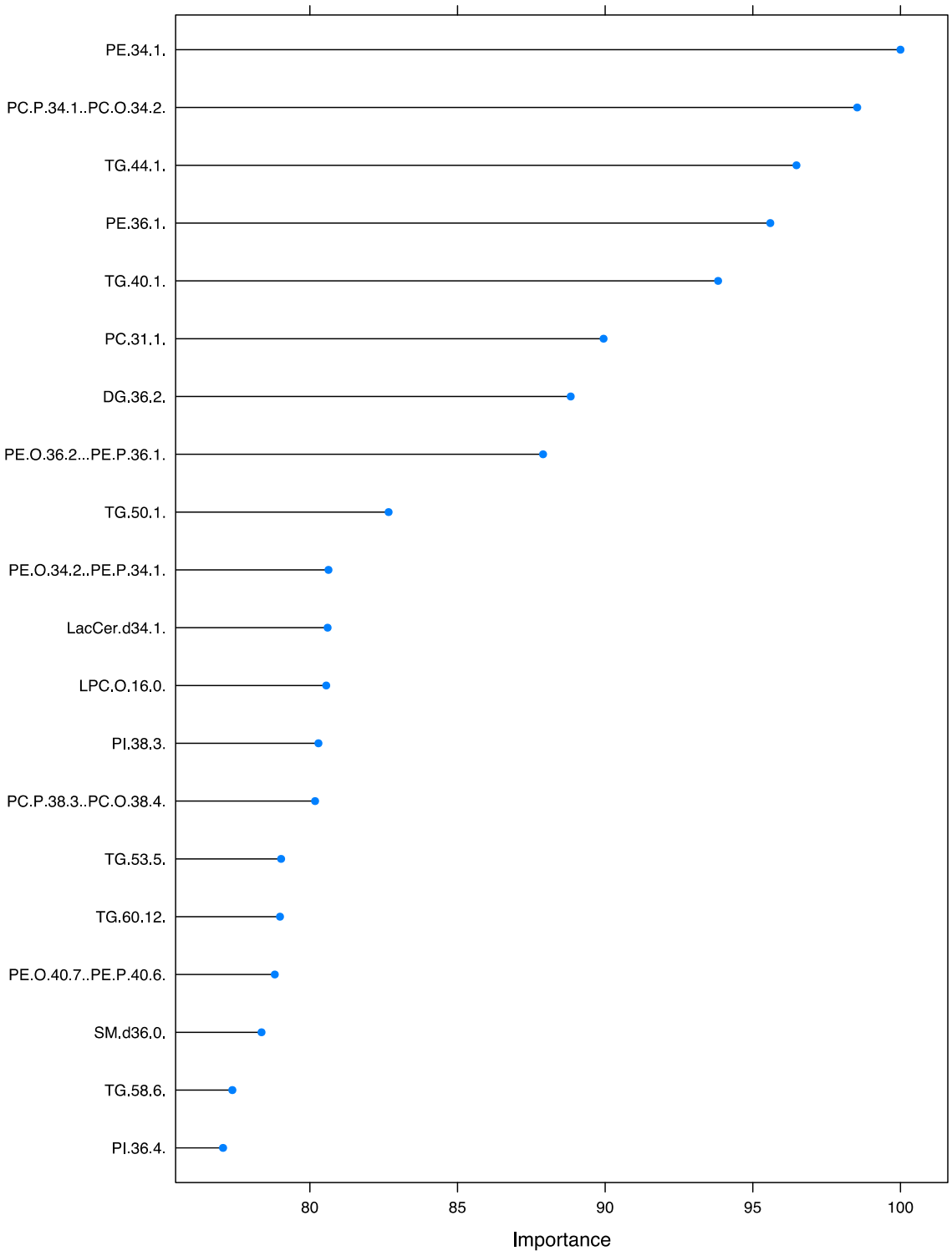
40
60
80
100
Importance

5F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LPC.O.16.0.
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PC.P.38.3..PC.O.38.4.
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TG.60.12.
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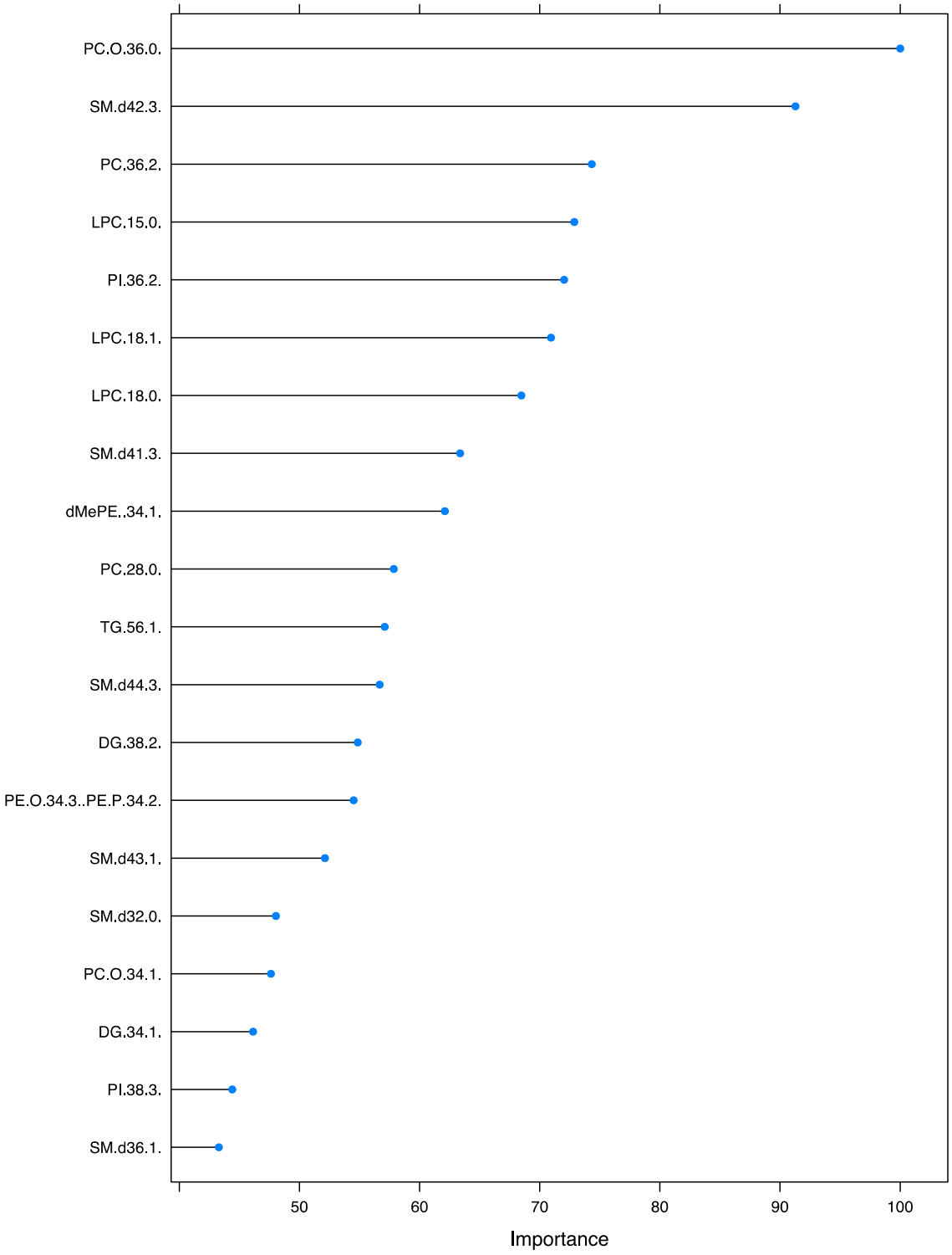
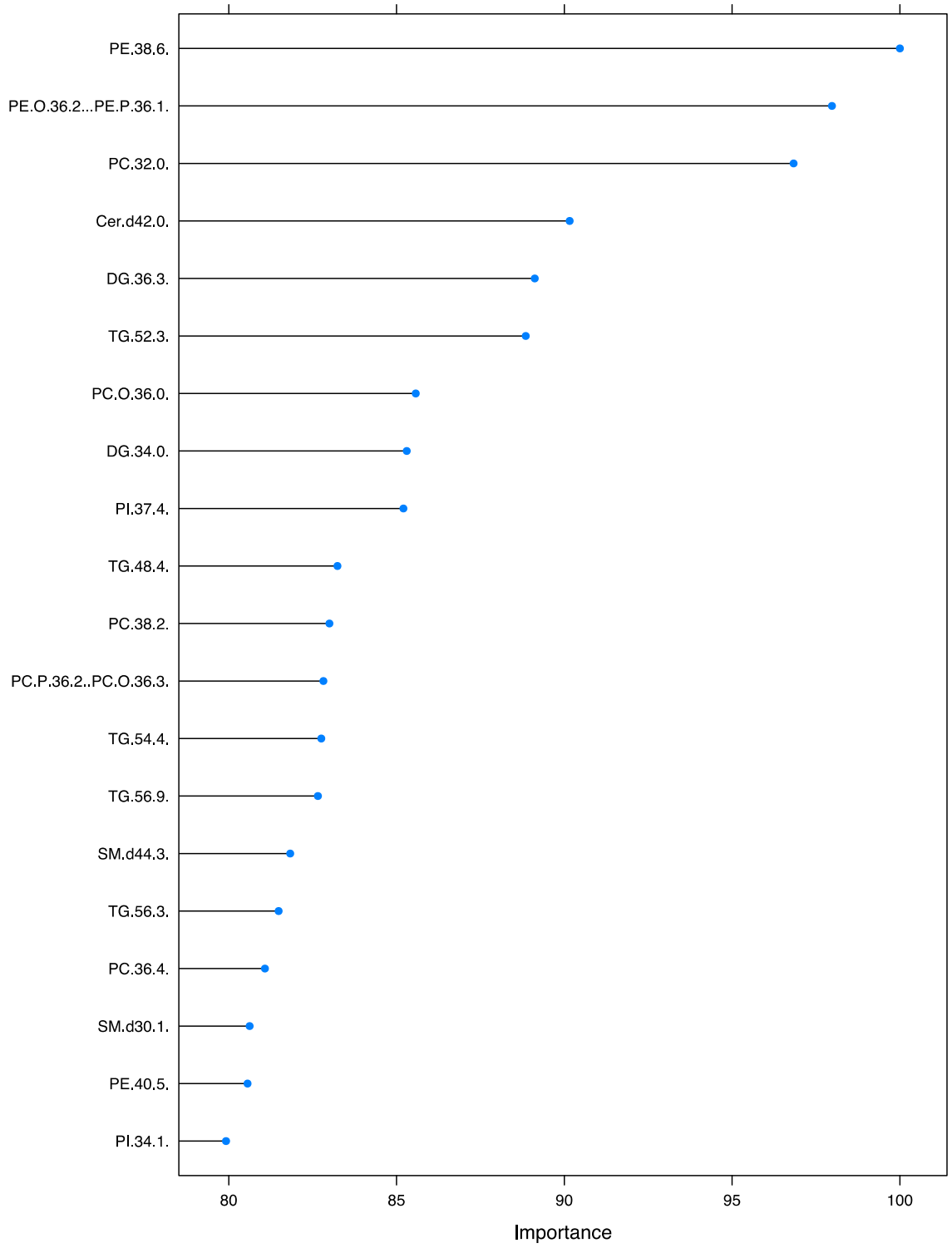
PI.36.4.
80
85
90
95
100
Importance

5G


PC.O.36.0.
SM.d42.3.
PC.36.2.
LPC.15.0.
PI.36.2.
LPC.18.1.
LPC.18.0.
SM.d41.3.
dMePE..34.1.
PC.28.0.
TG.56.1.
SM.d44.3.
DG.38.2.
PE.O.34.3..PE.P.34.2.
SM.d43.1.
SM.d32.0.
PC.O.34.1.
DG.34.1.
PI.38.3.
SM.d36.1.
50
60
70
80
90
100
Importance

5H



Variable Importance (VI) of the top 20 lipids following multivariate analyses. **A).** Controls vs MCI Aβ+ using PLS-DA; **B)** Controls vs MCI Aβ+ using RF; **C)** MCI Aβ- vs MCI Aβ+ using PLS-DA; **D)** MCI Aβ- vs MCI Aβ+ using RF; E) CERAD composite T at T1 using PLS

regression; **F)** CERAD composite T at T1 using RF regression**; G)** ΔCERAD composite T using PLS regression; **H)** ΔCERAD composite T using RF regression.