Supplementary Methods

1. Subjects

This study used plasma samples from EMIF-AD Multimodal Biomarker Discovery (EMIF-AD MBD) study [1]. EMIF-AD MBD is a cross-cohort study consisting of collated data from 11 European cohorts that aims to discover novel diagnostic and prognostic markers for AD-type dementia by performing analyses in multiple biomarker modalities [1]. This cross-cohort study includes a total of 1221 individuals across the cognitive spectrum: normal cognition (NC), Mild Cognitive Impairment (MCI) and AD-type dementia. Individuals were selected from prospective cohort studies based on the availability of plasma, DNA and CSF samples and MRI scans. Exclusion criteria for the EMIF-AD MBD study were the presence of neurological, psychiatric or somatic disorders that could cause cognitive impairment [1].

For the current study, we selected all participants from whom plasma samples were available for metabolomics analyses (n = 593). Participants were included from three multicenter studies: DESCRIPA (n = 16) [2], EDAR (n = 83) [3], PharmaCog (n = 40) [4], and eight single center studies: Amsterdam (n = 146) [5], Antwerp (n =1 30) [6], San Sebastian GAP (n = 40) [7], Gothenburg (n =22) [8], IDIBAPS (n =8) [9], Lausanne (n = 20) [10], Leuven (n = 53) [11], Barcelona-Sant Pau (n = 35) [12].

2. Clinical and cognitive data

The definition of NC was a normal performance on neuropsychological assessment (within 1.5 SD of the average for age, gender and education). MCI was defined as having cognitive complaints and performance below 1.5 SD of the average on at least one neuropsychological test but no dementia [13]. AD-type dementia diagnosis was

made based on a clinical diagnosis, using the National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria [14].

Mini Mental State Examination (MMSE) score [15] measuring an overall cognitive impairment was available for 590 participants at the time of blood donation and 405 participants at follow-up (the average follow-up length of 2.44 years). Rate of cognitive decline (ROD) was calculated using linear mixed effect models based on the longitudinal MMSE assessments (n=405). After covariate adjustment (age at sampling, gender, *APOE* ε4), the slope coefficient for each sample was derived, multiplied by -1 and then computed as z-score. The resulting values were defined as the ROD.

Neuropsychological tests measuring 5 different cognitive domains were also available at the time of blood donation; memory (-delayed, n=452; -immediate, n=537), language (n=572), attention (n=543), executive functioning (n=434), and visuoconstruction (n=346). One test for each cognitive domain was selected from each study and z-scores were computed based on local normative data when available, or published normative data from healthy controls otherwise. More detailed description on clinical diagnosis and assessment can be found in [1].

3. Amyloid and tau level measurements

Amyloid (Aβ) status was defined by the CSF $A\beta_{42/40}$ ratio of the central analyses (n=467). The ratio was derived from Aβ measurements using the 6E10 version of the MSD Abeta Triplex assay (Meso Scale Discovery, Rockville, MD). When no CSF was contributed for central analyses, the local CSF $A\beta_{42}$ value (n=73) or the standardized uptake value ratio (SUVR) on an amyloid-PET scan (n=53) was used [16].

Aβ measurements (Aβ z-scores) were calculated at each center (Central $A\beta_{42/40}$, local CSF $A\beta_{42}$, amyloid-PET) and later combined. The measures were obtained predominately in CSF (n=476), the local CSF $A\beta_{42}$ value (n=73) or the standardized uptake value ratio (SUVR) on an amyloid-PET scan (n=53). The Aβ z-scores were multiplied by -1 such that a positive score means more amyloid pathology. For phosphorylated tau (p-tau) and total tau (t-tau) the local measurements of each study were used, which we converted into z-scores within the group for which the same assay was used

For phosphorylated tau (p-tau) and total tau (t-tau) the local measurements of each study were used, which we converted into z-scores within the group for which the same assay was used. All central CSF analyses measuring phosphorylated tau (p-tau) and total tau (t-tau), were conducted using INNOTEST ELISA kits (Fujirebio, Ghent, Belgium) at the University of Gothenburg, Sweden.

The clinical design was explained in detail in the following publication [1]. Details on the amyloid and tau level measurements can be found in the supplementary methods 3.

2.4. Magnetic Resonance Imaging (MRI) analyses

Hippocampi volumes (left, right and sum) adjusted by intracranial volume were collected from 387 participants. Average cortical thickness across the whole brain and cortical thickness in AD signature regions as defined in [17] were available from 351 participants. Detailed information regarding data acquisition, processing, and quality control assessment has been described elsewhere [16].

2.5. Genetic analyses

Genome-wide SNP genotyping was performed using Global Screening Array (Illumina, Inc). *APOE* genotypes were defined using the rs429358 SNP. This SNP was determined through imputation based on the Global Screening Array, but was also directly genotyped locally. Participants were classified as *APOE* ε4 carriers (ε4+) or non-carriers (ε4-).

4. Metabolomics data acquisition and treatment

Metabolomics data for the current study was acquired by Metabolon Inc. (Morrisville, NC, USA). Relative levels of 883 plasma metabolites were measured in fasting blood samples using three different mass spectrometry methods. The first method utilized acidic, positive ionization conditions chromatographically optimized for hydrophilic compounds (UPLC-MS/MS positive polar). The second method used the same acidic positive ionization conditions but was chromatographically optimized for hydrophobic compounds (UPLC-MS/MS positive). The third method used negative ionization optimized conditions (UPLC-MS/MS negative). More details on the analytical method can be found in [17-19].

From the raw data, area counts for each metabolite in each sample were extracted. The extracted area counts were then normalized to correct for variation resulting from instrument inter-day tuning differences by the median value for each run-day, therefore, setting the medians to 1.0 for each run. This preserved variation between samples but allowed metabolites of widely different raw peak areas to be compared on a similar graphical scale. Metabolite levels below limit of quantification were replaced with 1 while metabolites with more than 20% missing were excluded from the further analysis. This step resulted in data reduction from 883 to 648 metabolites, this step removed 85

metabolites of drug origin leaving 2 in the data set. Subsequently, the metabolomics data was log transformed to allow the data to be normally distributed and then each metabolite was scaled to have a mean value of 0 and a standard deviation value of 1.

5. Statistical Analyses

Prior to statistical analyses, baseline characteristics were compared between diagnostic group using the Chi-square test for categorical variables and ANOVA for continuous variables (Table 1). To investigate the association of each metabolite with AD clinical variables, linear regression was applied for continuous AD variables (Aβ, p-tau, t-tau, cognition, and MRI measurements) while logistic regression was applied for categorical AD variables (diagnosis and *APOE4* alleles). Each regression model was adjusted for age at sampling, gender and presence of *APOE ε4*. For the *APOE* ε4 model, covariate adjustment was applied for age at sampling and gender. Adjustment for multiple testing was applied using a Bonferroni correction P-value $<7.72\times10^{-5}$ (= 0.05 / 648), where 648 is the number of metabolites tested against each AD clinical variable. All associations are reported as the change per one metabolite standard deviation (SD).

A schematic workflow of the primary data analysis used in this study can be found in the Supplementary Figure 1. All statistical analyses were performed using R Statistical Software (version 3.4.1). A circos plot [20] was generated using Perl based Circos algorithm (version 5.26.2).

4. Reference

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