



**Universiteit
Leiden**
The Netherlands

Circulating metabolites and general cognitive ability and dementia: Evidence from 11 cohort studies

Lee, S.J. van der; Teunissen, C.E.; Pool, R.; Shipley, M.J.; Teumer, A.; Chouraki, V.; ... ; Duijn, C.M. van

Citation

Lee, S. J. van der, Teunissen, C. E., Pool, R., Shipley, M. J., Teumer, A., Chouraki, V., ... Duijn, C. M. van. (2018). Circulating metabolites and general cognitive ability and dementia: Evidence from 11 cohort studies. *Alzheimer's And Dementia*, 14(6), 707-722.
doi:10.1016/j.jalz.2017.11.012

Version: Not Applicable (or Unknown)
License: [Leiden University Non-exclusive license](#)
Downloaded from: <https://hdl.handle.net/1887/66574>

Note: To cite this publication please use the final published version (if applicable).

Featured Article

Circulating metabolites and general cognitive ability and dementia: Evidence from 11 cohort studies

Sven J. van der Lee^a, Charlotte E. Teunissen^b, René Pool^c, Martin J. Shipley^d, Alexander Teumer^e, Vincent Chouraki^f, Debora Melo van Lent^{a,g}, Juho Tynkkynen^h, Krista Fischerⁱ, Jussi Hernesniemi^{h,j}, Toomas Hallerⁱ, Archana Singh-Manoux^{d,k}, Aswin Verhoeven^l, Gonneke Willemsen^c, Francisca A. de Leeuw^{b,m}, Holger Wagner^{n,o}, Jenny van Dongen^c, Johannes Hertel^p, Kathrin Budde^q, Ko Willems van Dijk^{r,s,t}, Leonie Weinhold^u, M. Arfan Ikram^{a,v}, Maik Pietzner^q, Markus Perola^{w,x}, Michael Wagner^{g,o}, Nele Friedrich^q, P. Eline Slagboom^y, Philip Scheltens^m, Qiong Yang^z, Robert E. Gertzen^{aa}, Sarah Egert^{bb}, Shuo Li^z, Thomas Hankemeier^{a,cc,dd}, Catharina E. M. van Beijsterveldt^c, Ramachandran S. Vasam^{ee,ff}, Wolfgang Maier^{g,o}, Carel F. W. Peeters^{gg}, Hans Jürgen Grabe^{o,hh}, Alfredo Ramirez^{n,o,ii,jj}, Sudha Seshadri^{kk,ll}, Andres Metspaluⁱ, Mika Kivimäki^d, Veikko Salomaa^w, Ayşe Demirkan^{a,s}, Dorret I. Boomsma^c, Wiesje M. van der Flier^{m,gg}, Najaf Amin^a, Cornelia M. van Duijn^{a,dd,*}

^aDepartment of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands

^bNeurochemistry Laboratory and Biobank, Department of Clinical Chemistry, VU University Medical Center, Amsterdam Neuroscience, Amsterdam, The Netherlands

^cDepartment of Biological Psychology, Vrije Universiteit Amsterdam, Neuroscience Campus Amsterdam, Amsterdam, The Netherlands

^dResearch Department of Epidemiology and Public Health, University College London, London, UK

^eInstitute for Community Medicine, University Medicine Greifswald, Greifswald, Germany

^fLille University, Inserm, CHU Lille, Institut Pasteur de Lille, U1167 - RID-AGE - Risk Factors and Molecular Determinants of Aging-Related Diseases, Labex Distalz, Lille, France

^gGerman Center for Neurodegenerative Diseases (DZNE), Bonn, Germany

^hUniversity of Tampere, Tampere, Finland

ⁱThe Institute of Genomics, University of Tartu, Tartu, Estonia

^jHeart Center, Tampere University Hospital, Tampere, Finland

^kInserm U1018, Centre for Research in Epidemiology and Population Health, Villejuif, France

^lCenter for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, The Netherlands

^mAlzheimer Center & Department of Neurology, Amsterdam Neuroscience, VU University Medical Center, Amsterdam, The Netherlands

ⁿDepartment of Psychiatry and Psychotherapy, University of Bonn, Bonn, Germany

^oDepartment for Neurodegenerative Diseases and Geriatric Psychiatry, University Hospital Bonn, Bonn, Germany

^pDepartment of Psychiatry and Psychotherapy, University Medicine Greifswald, Greifswald, Germany

^qInstitute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald, Germany

^rEindhoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The Netherlands

^sDepartment of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands

^tDepartment of Endocrinology and Metabolic Diseases, Leiden University Medical Center, Leiden, The Netherlands

^uDepartment of Medical Biometry, Informatics and Epidemiology, University Hospital Bonn, Bonn, Germany

^vDepartment of Radiology and Nuclear Medicine, Erasmus Medical Center, Rotterdam, The Netherlands

^wNational Institute of Health and Welfare, Helsinki, Finland

^xInstitute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland

^ySection of Molecular Epidemiology, Leiden University Medical Center, Leiden, The Netherlands

^zDepartment of Biostatistics, Boston University School of Public Health, Boston, MA, USA

^{aa}Division of Cardiovascular Medicine, Beth Israel Deaconess Medical Center, Boston, MA, USA

The authors have declared that no conflict of interest exists.

E-mail address: c.vanduijn@erasmusmc.nl

*Corresponding author. Tel.: +31-10-703-81-94; Fax: +31-10-7044657.

<https://doi.org/10.1016/j.jalz.2017.11.012>

1552-5260/© 2018 The Authors. Published by Elsevier Inc. on behalf of the Alzheimer's Association. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

^{bb}Department of Nutrition and Food Sciences, Nutritional Physiology, University of Bonn, Bonn, Germany

^{cc}Division of Analytical Biosciences, Leiden Academic Centre for Drug Research, Faculty of Science, Universiteit Leiden, Leiden, The Netherlands

^{dd}Translational Epidemiology, Faculty Science, Leiden University, Leiden, The Netherlands

^{ee}Department of Medicine, Boston University School of Medicine, Boston, MA, USA

^{ff}The Framingham Heart Study, Framingham, MA, USA

^{gg}Department of Epidemiology & Biostatistics, Amsterdam Public Health Research Institute, VU University Medical Center, Amsterdam, The Netherlands

^{hh}German Center for Neurodegenerative Diseases (DZNE), Rostock/Greifswald, Germany

ⁱⁱInstitute of Human Genetics, University of Bonn, Bonn, Germany

^{jj}Department of Psychiatry and Psychotherapy, University of Cologne, Cologne, Germany

^{kk}Department of Neurology, Boston University School of Medicine, Boston, MA, USA

^{ll}Glenn Biggs Institute of Alzheimer's and Neurodegenerative Diseases, University of Texas Health Sciences Center, San Antonio, TX, USA

Abstract

Introduction: Identifying circulating metabolites that are associated with cognition and dementia may improve our understanding of the pathogenesis of dementia and provide crucial readouts for preventive and therapeutic interventions.

Methods: We studied 299 metabolites in relation to cognition (general cognitive ability) in two discovery cohorts (N total = 5658). Metabolites significantly associated with cognition after adjusting for multiple testing were replicated in four independent cohorts (N total = 6652), and the associations with dementia and Alzheimer's disease (N = 25,872) and lifestyle factors (N = 5168) were examined.

Results: We discovered and replicated 15 metabolites associated with cognition including subfractions of high-density lipoprotein, docosahexaenoic acid, ornithine, glutamine, and glycoprotein acetyls. These associations were independent of classical risk factors including high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, glucose, and apolipoprotein E (*APOE*) genotypes. Six of the cognition-associated metabolites were related to the risk of dementia and lifestyle factors.

Discussion: Circulating metabolites were consistently associated with cognition, dementia, and lifestyle factors, opening new avenues for prevention of cognitive decline and dementia.

© 2018 The Authors. Published by Elsevier Inc. on behalf of the Alzheimer's Association. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Keywords:

Cognitive function; General cognitive ability; Alzheimer's disease; Dementia; Metabolites; Metabolomics; NMR; Lifestyle factors

1. Introduction

Cognitive function is an important determinant of health and well-being and a key component of the dementia spectrum, including Alzheimer's disease (AD), the most common cause of dementia [1]. Vascular dysfunction and metabolic dysregulation contribute to impairment in cognitive performance [2]. Clinical and population-based studies suggest a relationship of cognitive function with midlife hypertension, high blood levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides and glucose, and low levels of high-density lipoprotein cholesterol (HDL-C) [3–5]. The recent decrease in incidence of dementia in longitudinal studies has been attributed to improved control of vascular and metabolic factors [6–9]. These findings have fueled speculation that discovery of other circulating metabolites influencing cognition and future dementia may not only improve our understanding of the determinants of cognition but may also facilitate prevention through interventions on lifestyle factors and dedicated medication [10]. Previous studies have shown circulating metabolites in blood (e.g., lipoproteins, amino acids, fatty acids, and other small molecules) to be associated with cognitive function and conversion from

normal cognition to dementia or AD [11–17]. However, these studies were relatively small and findings have not been replicated [15,18], emphasizing the need for studies in large well-characterized populations where findings are replicated [10,19].

We performed a comprehensive metabolic analysis to study the role of circulating metabolites in cognitive function. Discovery of novel measures associated with cognitive function was performed in two large population-based studies in the Netherlands—the Rotterdam Study (RS) and the Erasmus Rucphen Family (ERF) study. We determined whether the associations were independent of known vascular and metabolic risk factors. Metabolites independently associated with cognition were replicated in independent cohort studies, and their relation to the risk of dementia and AD was validated in eight cohort studies. Finally, we assessed whether lifestyle factors, including dietary fish intake, smoking, and physical activity, were associated with the identified metabolites.

2. Methods

For a schematic overview of the analysis setup, see Fig. 1.

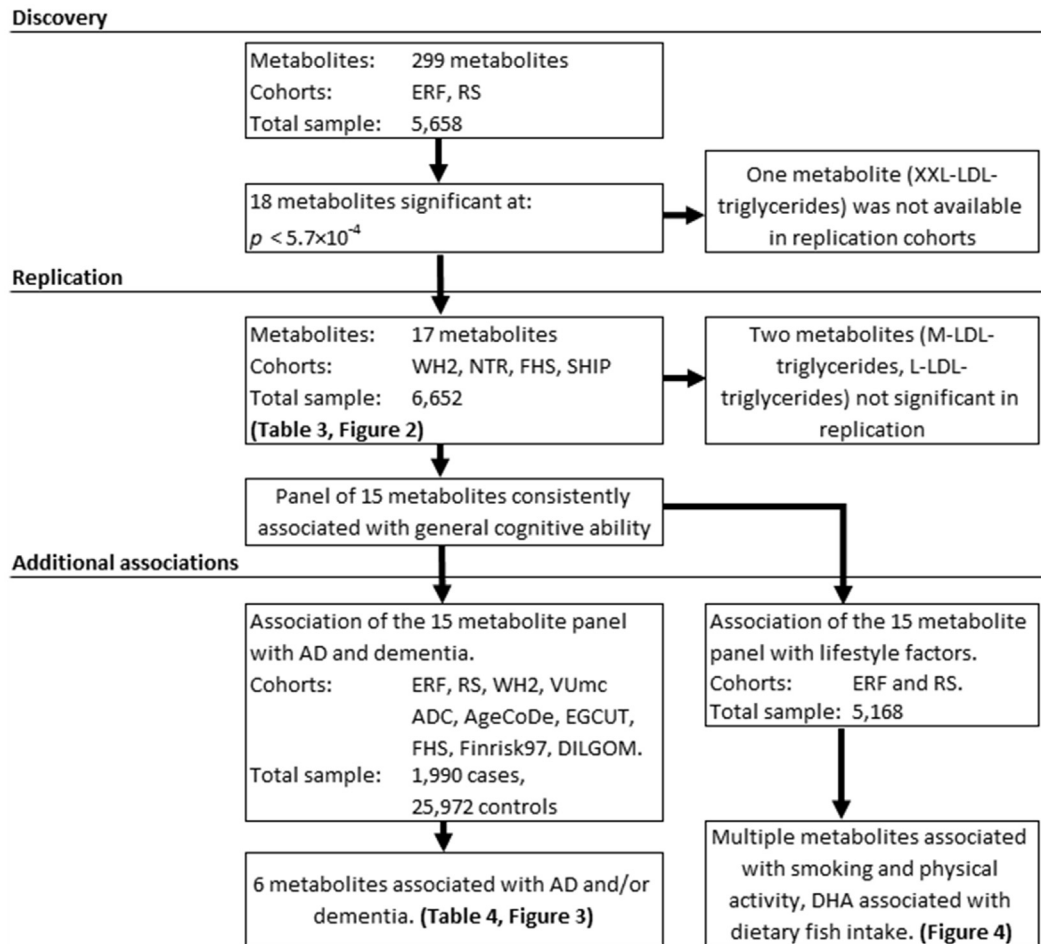


Fig. 1. Flowchart of analyses. Study names: ERF, RS, WHII, NTR, SHIP-Trend, FHS, VUmc ADC, Finrisk97, DILGOM, EGCUT, and AgeCoDe. Abbreviations: AgeCoDe, German Study on Ageing, Cognition, and Dementia; DHA, docosahexaenoic acid; EGCUT, Estonian Biobank; DILGOM, Dietary, Lifestyle, and Genetic determinants of Obesity and Metabolic Syndrome; ERF, Erasmus Rucphen Family; FHS, Framingham Heart Study; Finrisk97, National FINRISK Studies 1997; LDL, low-density lipoprotein; NTR, Netherlands Twin Register; RS, Rotterdam Study; SHIP-Trend, Study of Health in Pomerania-Trend; VUmc ADC, VUmc Amsterdam Dementia Cohort; WHII, Whitehall II.

2.1. Discovery and replication populations for research of cognitive function

Metabolomics profiling in multiple cohorts from the Netherlands was done as part of the BioBanking for Medical Research Infrastructure of the Netherlands (BBMRI) metabolomics consortium. These include the two discovery cohorts (ERF and RS). A short description of the cohort studies included in this article can be found in [Supplementary Table 1](#). ERF is a prospective family-based study (ERF, N = 2683) from the southwest of the Netherlands, and the RS is a prospective population-based cohort study that started in 1990 in Ommoord, a district of Rotterdam. In this analysis, we used the fourth wave of the baseline cohort (N = 2975). Replication cohorts included the Netherlands Twin Register (NTR, N = 338; also part of the BBMRI Metabolomics Consortium), the Whitehall II (WHII, N = 4612) study [20], the Framingham Heart Study (FHS, N = 2356), and the Study of Health in Pomerania-Trend (N = 944).

2.2. Cohorts for extrapolation to dementia and AD

Dementia and AD was assessed in eight cohorts; the ERF study, RS, a series of dementia patients and controls from the VUmc Amsterdam Dementia Cohort metabolically characterized as part of the BBMRI Metabolomics Consortium (N = 1303) [21], two cohorts used in the replication of cognitive findings (WHII = 4,612 and FHS = 2356), the National FINRISK Studies 1997 (Finrisk97; N = 7517), Dietary, Lifestyle, and Genetic determinants of Obesity and Metabolic Syndrome (DILGOM; N = 4788), the Estonian Biobank (EGCUT; N = 2572), and the German Study on Ageing, Cognition, and Dementia (AgeCoDe, N = 310).

2.3. Assessment of cognitive function and dementia

Participants underwent cognitive tests using a highly variable battery of assessments, which varied across studies; details on the cognitive tests used can be found in [Supplementary Table 2](#). Cognitive function tests were

assessed at the same time point in all studies. To enable meta-analyses of results from the heterogeneous set of tests efficiently, we constructed a general cognitive ability score

to capture information from a wide variety of cognitive tests reliably into a single cognitive measure [22,23]. General cognitive ability was calculated by principal component

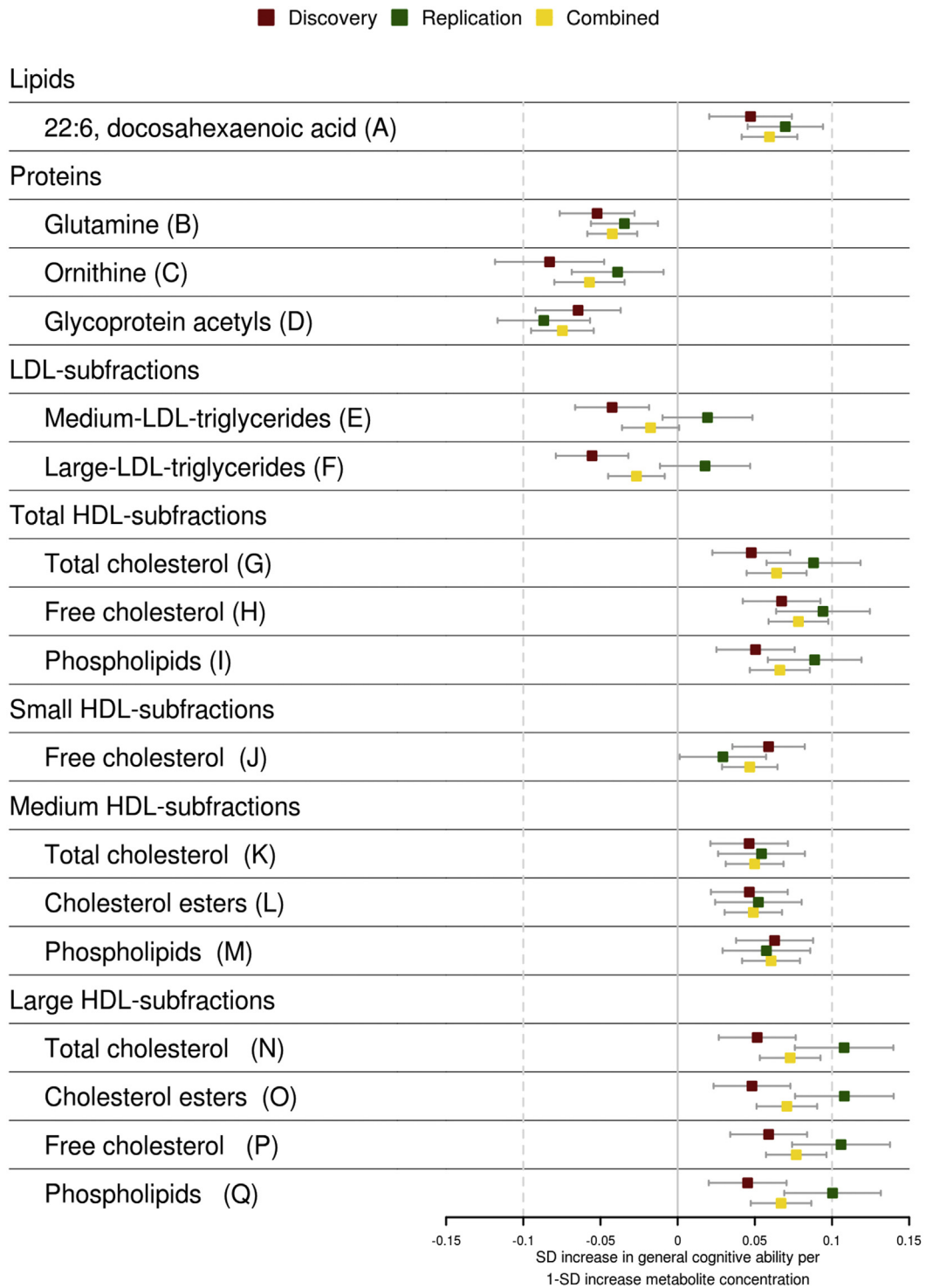


Fig. 2. Associations of metabolites with general cognitive ability. The standardized effect estimates on general cognitive ability of metabolites adjusted for age, sex, body mass index, and lipid-lowering medication use are shown. The estimates are shown for the discovery (red), replication (green), and the combined (yellow) analysis. Point estimates are shown as boxes with whiskers denoting the 95% confidence interval of the effect estimates. Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation.

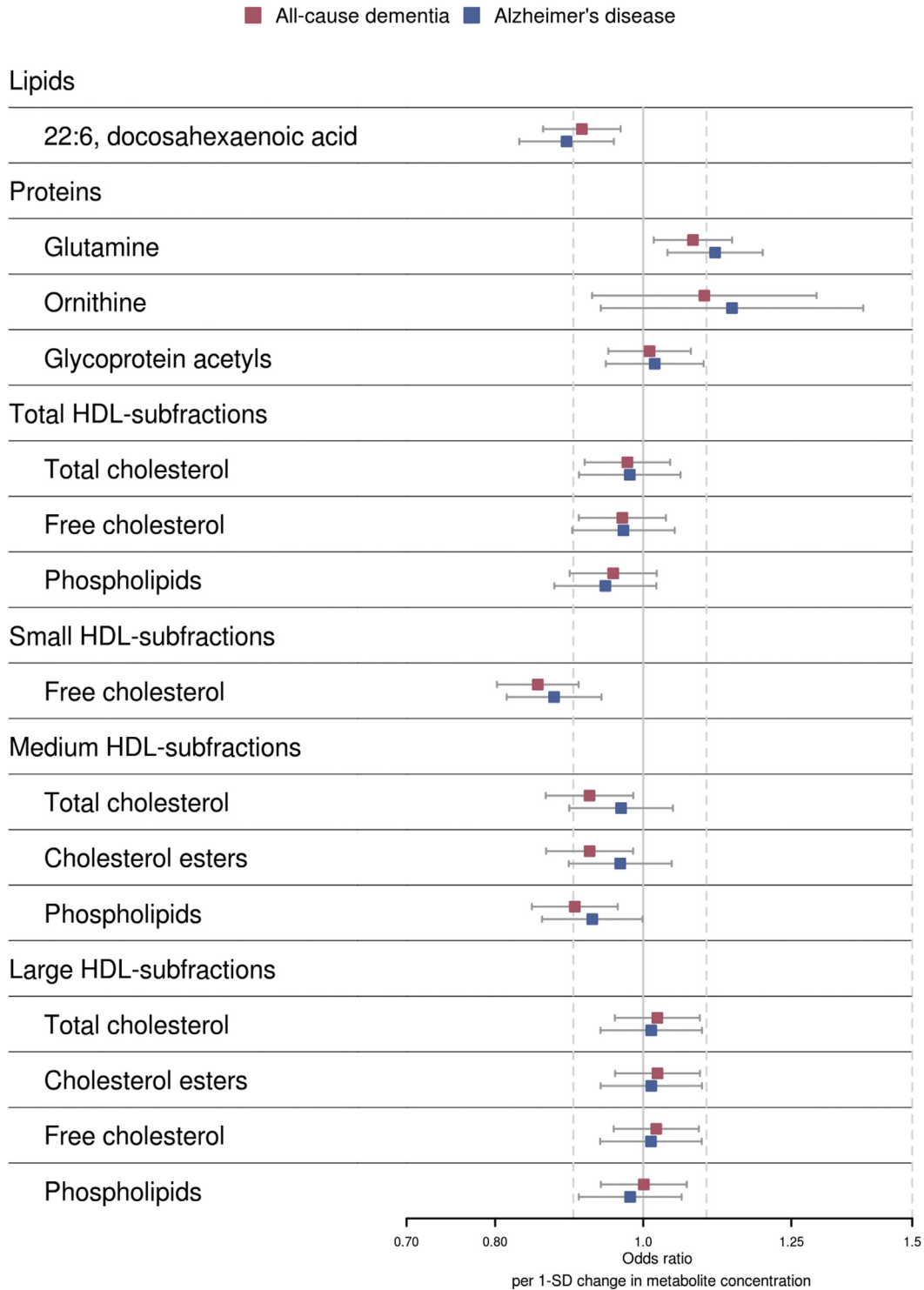


Fig. 3. Association of metabolites with all-cause dementia and Alzheimer's disease. The standardized OR of metabolites with all-cause dementia (red) and Alzheimer's disease (blue) shown as point estimates with whiskers denoting the 95% confidence interval of the OR. Associations shown are adjusted for age (at entry), sex, and if available body mass index and lipid-lowering medication. Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; OR, odds ratio; SD, standard deviation.

analysis of the different cognitive tests, the first principal component being the measure representing general cognitive ability [22,24,25]. General cognitive ability can be reliably estimated over the life course [26] and is very

similar when derived from different cognitive test batteries in the same individuals [22,23]. To ensure comparability for the general cognitive ability in our study, only studies that had cognitive tests covering at least three different

cognitive domains were included. The domains covered are shown in [Supplementary Table 3](#). General cognitive ability accounted for between 35% and 58% of variance in cognitive tests in various studies ([Supplementary Table 3](#)). Correlations between the individual test measures and the derived principal component (loadings) by study are shown in [Supplementary Table 3](#). In all studies, the general cognitive ability had a high correlation with multiple single cognitive measures, showing the factor was not driven by a single measure.

Details on the ascertainment of dementia and AD for the various cohorts can be found in [Supplementary Table 2](#). The diagnosis of dementia is based on continuous follow-up health records in the ERF, WHII, EGCUT, Finrisk97, and DILGOM. Studies that additionally used data on periodic visits to a research center were the RS, FHS, and AgeCoDe. The ascertainment of dementia and AD in the VUmc Amsterdam Dementia Cohort was done by clinical visits of participants.

2.4. Assessment of genetic and environmental factors

In the two discovery cohorts, ERF and RS, apolipoprotein E ε4 (*APOE* ε4) genotypes were determined by direct genotyping [27,28]. In both studies, lifestyle factors, including smoking (current vs. past and never smokers), physical activity (yes/no), and dietary fish (oil) intake [29] were ascertained using questionnaires as described previously [30,31]. Glucose, TC, HDL-C, LDL-C, and triglycerides were measured in mainly fasting blood samples by standard procedures [32,33]; further details are provided in [Supplementary Table 1](#). Multiple metabolites associated with smoking and physical activity, and docosahexaenoic acid (DHA) associated with fish intake ([Fig. 4](#)).

2.5. Assessment of blood metabolites

In the ERF and RS, the metabolic biomarkers were quantified from fasted ethylenediaminetetraacetic acid (EDTA) plasma samples using high-throughput proton nuclear magnetic resonance (NMR) metabolomics (Nightingale Ltd, Helsinki, Finland). This method provides simultaneous quantification of metabolites, that is, routine lipids, lipoprotein subclass profiling with lipid concentrations within 14 subclasses, fatty acid composition, and various low-molecular weight metabolites including amino acids, ketone bodies, and gluconeogenesis-related metabolites in molar concentration units. Details of the experimentation and applications of this NMR metabolomics platform have been described previously [34,35]. Metabolomics measurements of the ERF study further included two NMR experiments [35,36]. If a metabolite was measured in a study by multiple experiments, the experiment measuring the largest number of samples would be used. In total, 299 unique metabolite concentrations were measured in ERF; and of these, 242 metabolites were also available in the RS. The summary statistics of metabolomics measurements in the discovery cohorts are shown in [Supplementary Table 4](#). The cohorts used for replication of the cognitive findings and extrapolation to dementia used NMR-based platforms or mass spectrometry techniques ([Supplementary Table 1](#)). The Nightingale NMR platform was also used in NTR, VUmc, EGCUT, WHII, Finrisk97, and DILGOM. In NTR, additional NMR experiments [35,36] were performed, and again the experiment with the largest number of observations was used. Measurements of cognitive function and blood drawn for metabolite measurements were concurrent in all metabolite measurements from our discovery and 73.6% of the

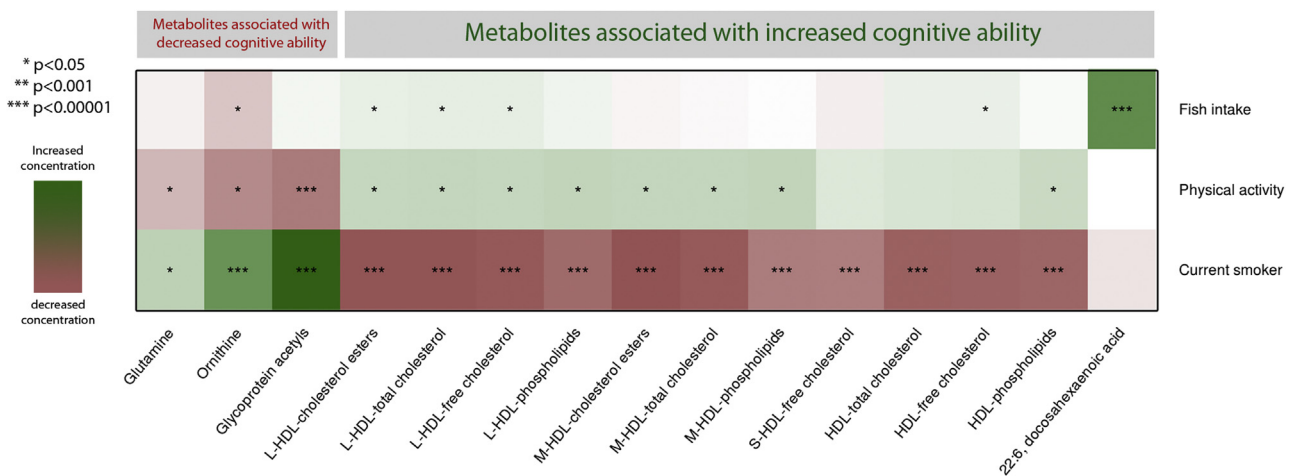


Fig. 4. Association of metabolites with lifestyle factors. Lifestyle factors including smoking (current vs. past and never smokers), physical activity (yes/no), and dietary fish (oil) intake. Metabolites are grouped based on their association with general cognitive ability and subtraction of HDL. Colors represent standardized effects estimates. Green shows the lifestyle factor associated with an increase in the metabolite concentration, and red shows the lifestyle factor associated with a decrease in the metabolite concentration. Significance of the associations is shown **P* < .05, ***P* < .001, and ****P* < 1 × 10⁻⁵. Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; L, large particles; M, medium particles; S, small particles.

samples in the replication cohorts (Supplementary Table 3). Samples used in replication and extrapolation were collected after overnight fasting; except for the samples at the VUmc Alzheimer Center and Finrisk97, which were nonfasting or “semifasting” (participants were instructed to fast for 4 hours before the scheduled examination). The summary statistics of metabolomics measurements in the cohorts used for the replication of the cognitive findings and the cohorts used for extrapolation to dementia are shown in Supplementary Table 5.

2.6. Statistical analyses

Histograms of classical blood measurements and metabolites in the discovery cohorts were visually inspected for non-normality, if necessary natural logarithmic or rank-transformations were applied (Supplementary Table 4). Individuals who had suffered from a stroke or who were diagnosed with dementia at the time of cognitive assessment were excluded. Linear regression analyses were used to assess the relation of standardized measures of TC, HDL-C, LDL-C, triglycerides, and glucose with general cognitive ability, adjusting for age, sex, lipid-lowering medication (yes/no), and body mass index (BMI) as covariates. The effect of *APOE* $\epsilon 4$ on general cognitive ability was assessed using an additive model. The association of 299 standardized metabolites with general cognitive ability was assessed using linear regression with age, sex, BMI, and lipid-lowering medication as covariates (model 1). To test if the identified associations were independent of the classically measured and frequently studied circulating markers, we ran a second model (model 2) where we additionally included TC, HDL-C, LDL-C, triglycerides, and glucose as covariates to model. Finally, we tested if the identified metabolites–general cognitive ability association were confounded by *APOE* $\epsilon 4$ (model 3).

Because metabolites are highly correlated, we used the method of Li and Ji [37] to correct for multiple testing. The method calculates the number of independent tests in correlated measures. In this study, testing 299 metabolites corresponded to 87 independent tests (P for significance = $0.05/87 = 5.7 \times 10^{-4}$). To assess the relation of metabolites found to be associated with cognitive function to incident dementia and AD, we used Cox proportional hazard models when data came from prospective studies. Again, we standardized the metabolite levels and adjusted for age (at entry), sex, BMI, and lipid-lowering medication. For VUmc Alzheimer Center, we used logistic regression adjusted for age and sex. The relations with incident dementia and AD were evaluated in a second model additionally adjusting for *APOE* $\epsilon 4$ genotypes.

In the discovery cohorts, we used linear regression analysis to study associations of lifestyle factors (smoking, physical activity, and fish [oil] consumption) with metabolites and cognitive function, adjusting for age, sex, BMI, and lipid-lowering medication. All analyses were performed in

R (version 3.2.1, 2015-06-18). Summary statistics by cohort were combined with inverse variance-weighted fixed-effects meta-analysis using the “*rmeta*” package (version 2.16). The association magnitudes are reported in units of standard deviation (SD) or odds ratio (OR) change per 1-SD increase in each metabolite [38,39] easing comparison of effects.

3. Results

Clinical characteristics of all cohorts analyzed in this study are provided in Table 1. Results of the association of general cognitive ability with baseline clinical characteristics in the discovery cohorts are shown in Table 2. As expected, general cognitive ability was higher in participants with higher education and was inversely associated with increasing age and the presence of *APOE* $\epsilon 4$ allele. Increased HDL-C was associated with higher general cognitive ability (0.034 SD higher general cognitive ability per 1 SD higher HDL-C concentration; $P = 6.4 \times 10^{-3}$), whereas fasting glucose levels were associated with lower cognitive ability (0.039 SD; $P = 2.2 \times 10^{-3}$).

3.1. The metabolic profile of general cognitive ability

We identified 18 metabolites that were significantly associated ($P < 5.9 \times 10^{-4}$ [model 1]) with general cognitive ability (listed as top 18 associations in Supplementary Table 6). Association results can be accessed through <http://bbmri.researchlumc.nl/atlas>. Of the 18 metabolites, XXL-LDL-triglycerides were not measured in the replication cohorts; therefore, 17 metabolites were tested for replication in independent cohorts (Supplementary Table 7 [model 1], $N_{\max} = 6652$). Of these 17, we found 15 to be associated with general cognitive ability in the replication cohorts ($P_{\text{replication}} < .05$, Table 3). Thirteen metabolites surpassed the more stringent Bonferroni corrected threshold for significance in the replication ($P_{\text{replication}} < 2.9 \times 10^{-3}$). Combining discovery and replication data (Table 3), 12 metabolites were associated with higher general cognitive ability and three were associated with lower general cognitive ability. The metabolites associated with increased higher cognitive ability include 11 HDL subfractions, the most significant being free cholesterol in HDL (0.078 SD; $P = 2.3 \times 10^{-15}$) and docosahexaenoic acid (DHA or 22:6[n-3]) an omega-3-fatty acid (0.060 SD; $P = 9.8 \times 10^{-11}$). The three metabolites that were associated with lower general cognitive ability include glycoprotein acetyls (-0.075 SD; $P = 5.4 \times 10^{-13}$), glutamine (-0.042 SD; $P = 2.8 \times 10^{-7}$), and ornithine (-0.057 SD; $P = 8.5 \times 10^{-7}$). Of the 15 metabolites significantly associated with general cognition, only two metabolites, HDL-C esters ($P_{\text{model2}} = 9.9 \times 10^{-3}$) and medium HDL TC ($P_{\text{model2}} = 7.9 \times 10^{-3}$), lost their significance in the combined analysis when additionally adjusting for glucose, TC, HDL-C, LDL-C, and triglycerides (Supplementary Table 7 [model 2]). However, adjustment did not result in a major change in effect estimates for these two metabolites,

Table 1
Baseline characteristics of all studied 11 cohorts

Variables	ERF	RS	WHII	NTR	SHIP-Trend	FHS	VUmc ADC	Finrisk97	DILGOM	EGCUT	AgeCoDe
Number of samples in cognitive analysis	2683	2505	4235	338	944	1508	-	-	-	-	-
Age (years)	48.9 ± 14.2	74.2 ± 6.2	55.8 ± 6.0	40.7 ± 12.4	50.1 ± 13.6	55.7 ± 9.8	64.1 ± 9.0	48.8 ± 13.5	52.3 ± 13.5	59.1 ± 12.4	84.1 ± 3.1
N-Women (%)	56.1	58.2	26.2	62.4	56.4	52.5	45.1	54.7	55.8	58.9	69.4
Education (1–4 scale)	2.1 ± 0.9	2.4 ± 0.9	2.0 ± 0.8	3.2 ± 0.8	2.4 ± 0.9	2.3 ± 0.6 *	5.0 ± 1.0 [†]	2.0 ± 0.8*	2.1 ± 0.8*	3.0 ± 0.8	-
Body mass index (kg/m ²)	27 ± 4.7	27.4 ± 4.1	25.9 ± 3.8	24.7 ± 4	27.4 ± 4.6	27.5 ± 4.9	25.3 ± 3.8	26.7 ± 4.6	27.2 ± 4.9	28 ± 5.1	25.6 ± 3.7
Lipid-lowering medication (%)	12.7	22.8	3.0	7.0	7.4	7.6	19.5	3.49	15.6	24	21.6
APOE ε4 carriers (%)	37.7	27.6	27.7	26.6	22.5	22.5	51.7	35.1	24	23.6	20.3
Diastolic blood pressure (mm Hg)	80 ± 10	79 ± 11	77 ± 10.3	76 ± 9.8	76.7 ± 10	75 ± 10	86 ± 11	83 ± 11.24	79 ± 11	82 ± 10	78 ± 8.0
Systolic blood pressure (mm Hg)	140 ± 20	152 ± 21	122 ± 15	124 ± 12	124 ± 16	126 ± 18	141 ± 19	136 ± 20	137 ± 20	134 ± 17	136 ± 15
Established blood measures											
TC (mmol/L)	5.6 ± 1.1	5.6 ± 1.0	5.9 ± 1.0	5.1 ± 1.1	5.5 ± 1.1	5.3 ± 1.0	4.9 ± 1.0	5.5 ± 1.1	5.3 ± 1.0	5.8 ± 1.1	5.8 ± 1.1
LDL-cholesterol (mmol/L)	3.7 ± 1.0	3.5 ± 0.9 [‡]	3.8 ± 0.9	3.1 ± 1.0	3.4 ± 0.9	3.3 ± 0.9	1.7 ± 0.5	3.5 ± 0.9 [‡]	3.2 ± 0.8 [‡]	2.2 ± 0.7	3.5 ± 1.0
HDL-cholesterol (mmol/L)	1.3 ± 0.4	1.5 ± 0.4	1.5 ± 0.4	1.4 ± 0.3	1.5 ± 0.4	1.3 ± 0.4	1.5 ± 0.4	1.39 ± 0.4	1.44 ± 0.4	1.7 ± 0.4	1.6 ± 0.4
Triglycerides (mmol/L)	1.3 ± 0.8	1.49 ± 0.7	1.3 ± 0.8	1.4 ± 0.7	1.4 ± 0.9	1.7 ± 1.4	1.4 ± 0.7	1.51 ± 1.1	1.43 ± 0.9	1.8 ± 1.1	1.4 ± 0.6
Glucose (mmol/L)	4.7 ± 1.1	5.9 ± 1.5	5.2 ± 1	5.4 ± 0.7	5.4 ± 0.6	5.6 ± 1.5	5.7 ± 1.7	4.61 ± 1.1	4.12 ± 0.8	4.6 ± 1.7	-
Dementia analysis											
Number of samples	1532	2010	4612	-	-	2356	1303	7517	4788	2572	310
Follow-up time (years)	11.3 ± 1.7	7.6 ± 3.6	16.7 ± 1.6	-	-	15.7 ± 5	-	9.67 ± 1.35	7.68 ± 0.9	7.03 ± 2.22	4.5 ± 1.8
Maximum follow-up	13.6	11.7	17.9	-	-	22.6	-	10	7.9	12.9	6.4
Number of AD cases	28	346	35	-	-	81	665	100	75	-	75
Number of dementia cases	39	506	114	-	-	110	917	141	81	41	82

Abbreviations: AD, Alzheimer's disease; AgeCoDe, German Study on Ageing, Cognition, and Dementia; APOE, apolipoprotein E; DILGOM, Dietary, Lifestyle, and Genetic determinants of Obesity and Metabolic Syndrome; EGCUT, Estonian Biobank; ERF, Erasmus Rucphen Family; FHS, Framingham Heart Study; Finrisk97, The National FINRISK Studies 1997; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NTR, Netherlands Twin Register; RS, Rotterdam Study; SHIP-Trend, Study of Health in Pomerania–Trend; TC, total cholesterol; VUmc ADC, VUmc Amsterdam Dementia Cohort; WHII, Whitehall II.

NOTE. For VUmc ADC, Finrisk97, DILGOM, EGCUT, and AgeCoDE the descriptive statistics are calculated based on the samples in the dementia analysis.

*Education in 1–3 scale.

[†]Education in 1–7 scale.

[‡]LDL-C estimated using the Friedewald estimation.

Table 2
Association of characteristics with general cognitive ability in discovery cohorts

Phenotype	ERF study			Rotterdam Study			Meta-analysis		
	Effect (\pm SE)	P value	N	Effect (\pm SE)	P value	N	Effect (\pm SE)	P value	N
Age	-0.042 (\pm 0.001)	2.1×10^{-280}	2699	-0.078 (\pm 0.003)	1.1×10^{-154}	2483	-0.046 (\pm 0.001)	$<1 \times 10^{-500}$	5182
Sex (male vs. female)	-0.020 (\pm 0.029)	0.49	2699	0.142 (\pm 0.035)	3.8×10^{-5}	2483	0.048 (\pm 0.022)	3.1×10^{-2}	5182
BMI	0.005 (\pm 0.003)	0.15	2694	-0.015 (\pm 0.004)	3.1×10^{-4}	2483	-0.003 (\pm 0.003)	0.30	5177
Education	0.410 (\pm 0.017)	1.4×10^{-117}	2699	0.277 (\pm 0.020)	1.5×10^{-41}	2483	0.355 (\pm 0.013)	$<1 \times 10^{-500}$	5182
APOE ϵ 4	-0.046 (\pm 0.028)	0.09	2342	-0.157 (\pm 0.035)	9.0×10^{-6}	2378	-0.088 (\pm 0.022)	5.2×10^{-5}	4720
Lipid-lowering medication	-0.131 (\pm 0.047)	5.8×10^{-3}	2690	0.013 (\pm 0.042)	0.76	2397	-0.050 (\pm 0.031)	0.11	5087
Classical blood measures									
TC	-0.011 (\pm 0.016)	0.50	2635	0.022 (\pm 0.019)	0.24	2481	0.003 (\pm 0.012)	0.8	5116
LDL-C*	-0.026 (\pm 0.016)	0.10	2621	0.016 (\pm 0.019)	0.41	2265	-0.009 (\pm 0.012)	0.45	4886
HDL-C	0.037 (\pm 0.016)	2.3×10^{-2}	2635	0.029 (\pm 0.019)	0.13	2401	0.034 (\pm 0.012)	6.4×10^{-3}	5036
Triglycerides	-0.014 (\pm 0.016)	0.36	2637	-0.017 (\pm 0.018)	0.35	2345	-0.015 (\pm 0.012)	0.19	4982
Glucose	-0.047 (\pm 0.017)	6.2×10^{-3}	2623	-0.029 (\pm 0.019)	0.12	2401	-0.039 (\pm 0.013)	2.2×10^{-3}	5024

Abbreviations: APOE, apolipoprotein E; BMI, body mass index; ERF, Erasmus Rucphen Family; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SD, standard deviation; SE, standard error; TC, total cholesterol.

NOTE. Multivariate analysis of general cognitive ability with age was adjusted for sex and with sex was adjusted for age. Association of general cognitive ability with BMI, educational level, APOE, and lipid-lowering medication use adjusted for age and sex. Associations of general cognitive ability with blood measures (TC, LDL-C, HDL-C, triglycerides, and glucose) were adjusted for age, sex, and lipid-lowering medication use. The association magnitudes are reported in units of SD change (\pm SE) per 1-SD increase in each metabolite [38,39].

*LDL-C in the Rotterdam Study was estimated using the Friedewald estimation.

suggesting an independent effect (Supplementary Fig. 1 [model 2]). Adjusting for APOE ϵ 4 did not change any of the 15 associations (Supplementary Table 7 [model 3] and Supplementary Fig. 1 [model 3]). In Box 1, we summarize the functions of the metabolites we found associated with general cognitive ability.

3.2. Association of the metabolic profile with dementia and AD

Next, we examined whether the 15 metabolites associated with general cognitive ability were associated with dementia. We compared (maximum) 1990 dementia patients, of whom 1356 were AD cases, with 23,882 controls. Six metabolites were associated with dementia, and three of these were also associated with AD ($P < .05$; Table 4, for all association results, see Supplementary Table 8 and Fig. 3). Free cholesterol in small HDL associated most significantly with a lower risk of dementia (OR = 0.85 per 1-SD increase in metabolite concentration; 95% CI = 0.80–0.91; $P = 6.3 \times 10^{-7}$) and AD (OR = 0.87; 95% CI = 0.81–0.94; $P = 2.3 \times 10^{-4}$). Other metabolites associated with a lower dementia risk were DHA (OR = 0.92; 95% CI = 0.86–0.97; $P = 3.4 \times 10^{-3}$; AD, $P = 1.5 \times 10^{-3}$) and subfractions of medium size HDL particles (phospholipids $P = 2.5 \times 10^{-3}$, TC $P = .025$, and cholesterol esters $P = .025$). Higher glutamine levels were associated with an increased risk of dementia (OR = 1.08; 95% CI = 1.02–1.15; $P = .011$) and AD (OR = 1.11; 95% CI = 1.04–1.20; $P = 3.0 \times 10^{-3}$). The association of free cholesterol in small HDL and DHA surpassed the more stringent Bonferroni corrected threshold for significance ($P < 1.5 \times 10^{-3}$). After additionally adjusting for the number of APOE ϵ 4 alleles, the associations of dementia and AD

with subfractions of medium size HDL particles were no longer significant ($P > .05$; Supplementary Table 8 and Supplementary Fig. 2).

3.3. Association of the metabolic profile with lifestyle factors

The analyses of the association of lifestyle factors with metabolites and general cognitive ability are shown in Supplementary Table 9 and summarized in Fig. 4. Fish (oil) intake was strongly associated with DHA blood concentrations ($P = 9.9 \times 10^{-53}$). Physical activity was associated with increased ($P < .05$) levels of metabolites that were associated with higher cognitive function (medium and large HDL subfractions) and decreased levels of metabolites that were associated with lower cognitive function (glycoprotein acetyls, ornithine, and glutamine). Smokers had decreased concentrations of all HDL subfractions associated with higher cognitive function and increased concentrations of metabolites associated with decreased cognitive function (Fig. 4).

4. Discussion

In this study, we discovered and replicated 15 metabolites associated with general cognitive ability. This metabolic profile includes subfractions of HDL, DHA, ornithine, glutamine, and glycoprotein acetyls. We show that metabolites in the profile are independent of classical cardiometabolic blood correlates of cognitive function. Of the 15 replicated metabolites, six were associated with dementia and three of these also with AD. Furthermore, we show that lifestyle factors, such as diet, smoking, and physical activity, have strong effects on metabolites in the profile.

Table 3
Association of metabolites with general cognitive ability

Metabolite	Discovery			Replication			Meta-analysis			I ²	P-I ²
	Effect (±SE)	P value	N	Effect (±SE)	P value	N	Effect (±SE)	P value	N		
HDL-free cholesterol	0.067 (±0.013)	1.5 × 10 ⁻⁷	4791	0.094 (±0.015)	1.2 × 10 ⁻⁹	4542	0.078 (±0.010)	2.3 × 10 ⁻¹⁵	9333	63	6.7 × 10 ⁻²
L-HDL-free cholesterol	0.059 (±0.013)	3.4 × 10 ⁻⁶	4793	0.106 (±0.016)	6.2 × 10 ⁻¹¹	4542	0.077 (±0.010)	1.5 × 10 ⁻¹⁴	9335	80	7.3 × 10 ⁻³
L-HDL-total cholesterol	0.052 (±0.013)	5.0 × 10 ⁻⁵	4792	0.108 (±0.016)	3.5 × 10 ⁻¹¹	4542	0.073 (±0.010)	3.5 × 10 ⁻¹³	9334	79	8.2 × 10 ⁻³
Glycoprotein acetyls	-0.064 (±0.014)	4.5 × 10 ⁻⁶	3778	-0.087 (±0.015)	1.4 × 10 ⁻⁸	4542	-0.075 (±0.010)	5.4 × 10 ⁻¹³	8320	0	0.60
L-HDL-cholesterol esters	0.048 (±0.013)	1.5 × 10 ⁻⁴	4792	0.108 (±0.016)	3.4 × 10 ⁻¹¹	4542	0.071 (±0.010)	1.6 × 10 ⁻¹²	9334	80	7.6 × 10 ⁻³
L-HDL-phospholipids	0.045 (±0.013)	4.2 × 10 ⁻⁴	4791	0.100 (±0.016)	3.3 × 10 ⁻¹⁰	4542	0.067 (±0.010)	2.2 × 10 ⁻¹¹	9333	78	9.7 × 10 ⁻³
HDL-phospholipids	0.050 (±0.013)	9.5 × 10 ⁻⁵	4790	0.089 (±0.015)	9.8 × 10 ⁻⁹	4542	0.066 (±0.010)	2.5 × 10 ⁻¹¹	9332	68	4.3 × 10 ⁻²
HDL-total cholesterol	0.048 (±0.013)	2.1 × 10 ⁻⁴	4796	0.088 (±0.016)	1.5 × 10 ⁻⁸	4542	0.064 (±0.010)	9.8 × 10 ⁻¹¹	9338	66	5.4 × 10 ⁻²
22:6, docosahexaenoic acid	0.047 (±0.014)	5.4 × 10 ⁻⁴	3772	0.070 (±0.012)	2.1 × 10 ⁻⁸	5480	0.060 (±0.009)	9.8 × 10 ⁻¹¹	9252	67	4.6 × 10 ⁻²
M-HDL-phospholipids	0.063 (±0.013)	8.2 × 10 ⁻⁷	4799	0.057 (±0.014)	7.2 × 10 ⁻⁵	4542	0.060 (±0.010)	2.5 × 10 ⁻¹⁰	9341	0	0.48
M-HDL-total cholesterol	0.046 (±0.013)	3.0 × 10 ⁻⁴	4799	0.054 (±0.014)	1.5 × 10 ⁻⁴	4542	0.050 (±0.010)	1.8 × 10 ⁻⁷	9341	0	0.62
M-HDL-cholesterol esters	0.046 (±0.013)	2.5 × 10 ⁻⁴	4799	0.052 (±0.014)	2.6 × 10 ⁻⁴	4542	0.049 (±0.009)	2.4 × 10 ⁻⁷	9341	0	0.63
Glutamine	-0.052 (±0.012)	2.5 × 10 ⁻⁵	4715	-0.034 (±0.011)	1.8 × 10 ⁻³	6652	-0.042 (±0.008)	2.8 × 10 ⁻⁷	11,367	74	9.8 × 10 ⁻³
S-HDL-free cholesterol	0.059 (±0.012)	8.4 × 10 ⁻⁷	4796	0.029 (±0.014)	4.0 × 10 ⁻²	4542	0.047 (±0.009)	3.5 × 10 ⁻⁷	9338	0	0.55
Ornithine	-0.083 (±0.018)	4.5 × 10 ⁻⁶	2228	-0.039 (±0.015)	1.0 × 10 ⁻²	2750	-0.057 (±0.012)	8.5 × 10 ⁻⁷	4978	43	0.18
L-LDL-triglycerides	-0.055 (±0.012)	3.7 × 10 ⁻⁶	4797	0.018 (±0.015)	2.3 × 10 ⁻¹	4542	-0.027 (±0.009)	4.2 × 10 ⁻³	9339	90	3.6 × 10 ⁻⁵
M-LDL-triglycerides	-0.042 (±0.012)	5.1 × 10 ⁻⁴	4800	0.019 (±0.015)	1.9 × 10 ⁻¹	4542	-0.018 (±0.009)	6.3 × 10 ⁻²	9342	85	1.3 × 10 ⁻³

Abbreviations: *APOE*, apolipoprotein E; HDL, high-density lipoprotein; I², measure for heterogeneity in the meta-analysis in percent; LDL, low-density lipoprotein; L, large particles; M, medium particles; P-I², P value for heterogeneity; S, small particles; SD, standard deviation; SE, standard error.

NOTE. Glycoprotein acetyls are mainly α -1-acid glycoprotein. The association magnitudes are reported in units of SD change (±SE) per 1-SD increase in each metabolite [38,39]. Shown associations of the metabolites with general cognitive ability are adjusted for age, sex, body mass index, and lipid-lowering medication.

Box 1**Description of pathways of metabolites in the context of cognitive function****High-density lipoprotein subfractions**

The specific lipoprotein subfractions could point to the specific functions of lipoprotein subfractions. High-density lipoprotein (HDL) fractions are well known to be individually tasked for different functions across lipid metabolism, inflammation, anti-oxidation, and host defense [40,41]. In addition, specific protein pairs on specific HDL subspecies exist that maintain stable compositions [42]. Previous research reported links between HDL cholesterol profiles and changes in vascular health with plaque accumulation in arteries of the brain, damage to the blood brain barrier [43], and occurrence of thrombosis. All are possibly leading to progressive vascular brain damage resulting in loss of white matter microstructural organization.

Docosahexaenoic acid

Docosahexaenoic acid (DHA) levels in blood are highly associated with omega-3-fatty acid intake through diet [39], and it cannot be de novo synthesized in the brain and is therefore actively transported over the blood-brain barrier through the Mfsd2a [44,45]. DHA is essential for normal brain development in early life and is frequently associated with cognition [46]. High intake might also be beneficial in late life as DHA and fish oil intake associated with less Alzheimer's disease pathology [47]. The evidence of the attributed beneficial effects of DHA on the brain in literature is inconsistent [48].

Glutamine

In the brain, glutamine is not only used for energy production and protein synthesis, as in other cells, but is also an essential precursor for biosynthesis of amino acid neurotransmitters. It is involved in the glutamine-glutamate/GABA cycle, a well-studied concept in excitatory signaling in the brain [49]. The cycle involves transfer of glutamine from astrocytes to neurons and neurotransmitter glutamate or GABA from neurons to astrocytes. The leading opinion in the field is that in the brain an excess of glutamate, excitotoxicity, is seen as detrimental and glutamine in the brain as beneficial [50].

Glycoprotein acetyls

The measured glycoprotein acetyls is mainly α -1-acid glycoprotein (AGP) [34], also called orosomucoid, which is an acute phase plasma α -globulin glycoprotein. The protein is widely studied and has previously been found to predict 10-year mortality [51]. Increased plasma levels of glycoprotein acetyls as reaction to various diseases (cancer and inflammatory diseases) or following trauma (surgery) might explain the association with increased mortality and could partially explain the association with general cognitive ability as chronic diseases decrease cognitive abilities. Another function of AGP is to carry mainly neutrally charged medications in blood, for example, antidepressants [52]. The plasma concentration of AGP is relatively low, and there is only one drug-binding site in each AGP molecule [53], leading to lower antidepressant response in higher AGP concentrations [54].

Ornithine

Ornithine as a non-proteinogenic amino acid is an important intermediate product in arginine degradation and urea cycle. Hyperornithinemia is also the biochemical hallmark of an inherited metabolic disease, hyperornithinemia-hyperammonemia-homocitrullinuria syndrome [55]. This disease is clinically characterized by mental retardation whose pathogenesis is still poorly known.

The most interesting metabolite in the profile is DHA, a long-chain omega-3 polyunsaturated fatty acid. As the largest cross-sectional study to date studying DHA in rela-

tion to cognitive function, the present study showed compelling evidence that DHA levels in blood were associated with higher cognitive function ($P = 9.8 \times 10^{-11}$). This finding is

Table 4
Metabolite concentrations associated with dementia and AD

Metabolite	AD				Dementia			
	OR	P value	N cases	N total	OR	P value	N cases	N total
S-HDL-free cholesterol	0.87 [0.81–0.81]	2.3×10^{-4}	1276	22,880	0.85 [0.80–0.80]	4.1×10^{-7}	1881	25,868
M-HDL-phospholipids	0.93 [0.86–0.86]	4.7×10^{-2}	1276	22,884	0.90 [0.85–0.85]	1.8×10^{-3}	1881	25,872
22:6, docosahexaenoic acid	0.89 [0.83–0.83]	1.5×10^{-3}	1334	22,466	0.91 [0.86–0.86]	1.9×10^{-3}	1938	25,417
Glutamine	1.11 [1.04–1.04]	3.1×10^{-3}	1356	25,181	1.08 [1.02–1.02]	1.3×10^{-2}	1990	25,640
M-HDL-cholesterol esters	0.97 [0.89–0.89]	0.38	1276	22,884	0.92 [0.86–0.86]	1.6×10^{-2}	1881	25,872
M-HDL-total cholesterol	0.97 [0.89–0.89]	0.40	1276	22,884	0.92 [0.86–0.86]	1.6×10^{-2}	1881	25,872

Abbreviations: AD, Alzheimer's disease; BMI, body mass index; HDL, high-density lipoprotein; L, large; M, medium; OR, odds ratio for the increase or decrease in AD or dementia risk per 1-SD increase of metabolite concentration.

NOTE. Sorted by the P values for dementia. Combined results from Cox proportional hazard models and logistic regression models are presented as OR. Associations shown are adjusted for age (at entry), sex, and if available BMI and lipid-lowering medication. N total is the sum of cases and controls.

in line with many previous studies, summarized by Cederholm et al. [46], suggesting a relation between nutritive DHA intake, or fish (oil) intake as its proxy, and better cognition. Blood levels of DHA are raised by eating fat fish, as also in our study. DHA from diet is most likely actively transported over the blood-brain barrier by Mfsd2a [44,56], where it is abundant in gray matter [57] and found in lower concentrations in brains of individuals with AD [58]. We showed for the first time that DHA in blood was associated with a lower risk of AD and dementia, using blood measures of DHA in up to 22,887 individuals. Taken together, our study implies that high levels of DHA could be beneficial for cognitive function, potentially also reducing the risk of dementia and AD.

Beyond the association of high HDL-C with better cognitive function [3–5], the present study points toward a role of cholesterol, free cholesterols, and phospholipids in small, medium, and large subclasses of HDL. However, current knowledge of the functions of HDL subclasses is limited; thus, we can only speculate on the pathways through which the metabolites that we observed exert their effect on cognitive function [59]. Phospholipids could have a direct effect as they are the main constituents of neuronal membrane structures, such as presynaptic and postsynaptic membranes, and neuronal membrane degeneration has been linked to synapse loss in AD [60]. Possibly, circulating phospholipids and free cholesterols in HDL form a buffer to repair damaged membranes. This is supported by the observation that both AD patients and patients with mild cognitive impairment have lower circulating levels of nutrients involved in phospholipid synthesis in blood and cerebrospinal fluid [61]. Alternatively, the free cholesterols in the phospholipid layer of HDL tag the presence of other important proteins that are transported to or are disposed from the brain. HDL contains up to 95 proteins and lipids that may segregate into distinct subclasses of HDL and lead to subclass-specific effects [42,62]. Regions in membranes of both neurons and astrocytes [63], where HDL-related free cholesterols, sphingomyelins, and free fatty acids (such as DHA) concentrate, are called lipid rafts. Changes in lipid raft composition may be an early marker of neurodegenerative diseases [64]. A hypothesis that requires further study is that increased free cholesterols in (small) HDL and DHA in blood affects lipid raft quantity, composition, or cell-signaling leading to beneficial effects on the brain.

In our study, levels of glycoprotein acetyls, mainly α -1-acid glycoprotein (also known as orosomucoid, an acute phase protein), were associated with lower cognitive function, smoking, and physical activity. Glycoprotein acetyl concentration has been shown to be a strong predictor of 10-year mortality [51,65]. A major genetic determinant of glycoprotein acetyl levels in blood is located close to the gene coding for haptoglobin (*HP*) [35]. This protein may link our findings of HDL subfractions to that of glycoprotein acetyls, as the *HP* protein has been found in specific HDL subfractions [62]. Furthermore, the *HP* gene was previously

associated with the risk of cognitive impairment in type 2 diabetes with poor glycemic control [66].

Two nonessential amino acids that were associated with lower cognitive function were ornithine, which is part of the urea cycle, and glutamine. Ornithine accumulation causes hyperornithinemia-hyperammonemia-homocitrullinuria syndrome [55], a disease with a currently poorly known pathogenesis, which is clinically characterized by mental retardation [67]. Glutamine and its closely related neurotransmitter glutamate have been found to be differentially expressed in brains of AD patients [49]. In the brain, glutamate is considered harmful [49], and our population-based studies suggest that in the circulation, glutamine is associated with lower cognition. Both ornithine and glutamine are interesting targets for further studies.

A major strength of the present study is the large sample size, both in the discovery and replication. To our knowledge, this is the largest study exploring the association of a large array of blood-based metabolites with general cognitive ability to date. Other strengths are the similar methods across studies used to determine metabolites and the use of general cognitive ability to harmonize the studied cognitive outcome [23]. We chose to analyze the associations of metabolites with cognitive ability in the largest sample size available, accepting that subtle differences in cognitive testing, metabolite measuring, study design, and populations would introduce heterogeneity of effects and then followed by a replication in independent samples; this approach is modeled to the standard approach followed in genome-wide association studies [68]. A potential limitation of our cross-sectional study of cognition is that we cannot determine causality of the association with circulating metabolites. However, in our extrapolation to dementia and AD, we mostly studied incident cases with metabolites measured before the disease onset, suggesting that at least the six dementia-associated metabolites are most likely in a causal pathway. We note that the associations of the HDL subfractions associated with dementia were attenuated by the *APOE* ϵ 4 genotype, suggesting they could be in the causal pathway of *APOE* ϵ 4 to dementia or the associations found are pleiotropic effects of *APOE* ϵ 4. Last but not least, we did not adjust for education because cognition and education are highly correlated [69] if measured at the same time. In fact, there is still debate on whether education determines cognitive ability [26,70] or vice versa [71,72]. In fact, there is a very high genetic correlation between educational attainment and cognitive ability ($R^2 = 0.55$ based on linkage disequilibrium (LD) score regression) [22,73,74]. This shared genetic background is probably the primary reason for the high correlation between education and cognitive ability [69,75]. Given the high genetic correlation, we decided that adjusting for education as a covariate in the model would lead to overadjustment and ultimately false-negative findings in the study.

In conclusion, we discovered and replicated the relation of 15 metabolites in blood to cognitive function in cognitively healthy individuals. We found that six metabolites

were associated with dementia and three with AD. The association of lifestyle factors to the metabolites associated with cognitive ability and dementia opens new avenues for targeted prevention.

Acknowledgments

None of the funders had any role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication.

This work was performed within the framework of the BBMRI Metabolomics Consortium funded by BBMRI-NL, a research infrastructure financed by the Dutch government (NWO, grant nr 184.021.007 and 184033111). This work is funded by the European Union's Horizon 2020 research and innovation programme as part of the Common mechanisms and pathways in Stroke and Alzheimer's disease (CoSTREAM) project (www.costream.eu, grant agreement No 667375); the Netherlands Organisation for Health Research and Development (ZonMw) as part of the Joint Programming for Neurological Disease (JPND) project PERADES (Defining Genetic, Polygenic and Environmental Risk for Alzheimer's Disease using multiple powerful cohorts, focused Epigenetics and Stem cell metabolomics - grant number 733051021); the European Union Innovative Medicine Initiative (IMI) programme under grant agreement No 115975 as part of the Alzheimer Disease Apolipoprotein Pathology for Treatment Elucidation and Development (ADAPTED, <https://www.imi-adapted.eu>) and the European Union's Horizon 2020 research and innovation programme Marie Skłodowska-Curie Research and Innovation Staff Exchange (RISE) under the grant agreement No 645740 as part of the Personalized pREvention of Chronic DIseases (PRECeDI) project.

The Erasmus Rucphen Family (ERF) has received funding from the Centre for Medical Systems Biology (CMSB) and Netherlands Consortium for Systems Biology (NCSB), both within the framework of the Netherlands Genomics Initiative (NGI)/Netherlands Organization for Scientific Research (NWO). The ERF study is also a part of EUROSPAN (European Special Populations Research Network; FP6 STRP grant number 018947 [LSHG-CT-2006-01947]); European Network of Genomic and Genetic Epidemiology (ENGAGE) from the European Community's Seventh Framework Programme (FP7/2007-2013)/grant agreement HEALTH-F4-2007-201413; "Quality of Life and Management of the Living Resources" of fifth Framework Programme (no. QLG2-CT-2002-01254); FP7 project EUROHEADPAIN (nr 602633), the Internationale Stichting Alzheimer Onderzoek (ISAO); the Hersenstichting Nederland (HSN). Metabolomics measurements of ERF have been funded by Biobanking and Biomolecular Resources Research Infrastructure (BBMRI)-NL (184.021.007). A.D. is supported by a Veni grant (2015) from ZonMw. The ERF-follow up study is funded by Cardio-

Vasculair Onderzoek Nederland (CVON 2012-03). We are grateful to all study participants and their relatives, general practitioners, and neurologists for their contributions and to P. Veraart for her help in genealogy, J. Vergeer for the supervision of the laboratory work, both S.J.v.d.L. and A. van der Spek for collection of the follow-up data, and P. Snijders, M.D., for his help in data collection of both baseline and follow-up data.

The Rotterdam Study is supported by the Erasmus MC University Medical Center and Erasmus University Rotterdam; The Netherlands Organisation for Scientific Research (NWO); The Netherlands Organisation for Health Research and Development (ZonMw); the Research Institute for Diseases in the Elderly (RIDE); The Netherlands Genomics Initiative (NGI); the Ministry of Education, Culture and Science; the Ministry of Health, Welfare and Sports; the European Commission (DG XII); and the Municipality of Rotterdam. The contribution of inhabitants, general practitioners, and pharmacists of the Ommoord district to the Rotterdam Study is gratefully acknowledged. Metabolomics measurements were funded by Biobanking and Biomolecular Resources Research Infrastructure (BBMRI)-NL (184.021.007).

The Whitehall II study is supported by the Medical Research Council (K013351), the British Heart Foundation, and the National Institute on Aging (R01 AG013196).

Netherlands Twin Register (NTR): Funding was obtained from the Netherlands Organization for Scientific Research (NWO) and MagW/ZonMw grants 904-61-090, 985-10-002, 904-61-193,480-04-004, 400-05-717, Addiction-31160008, Middelgroot-911-09-032, Spinozapremie 56-464-14192, Biobanking and Biomolecular Resources Research Infrastructure (BBMRI-NL, 184.021.007); VU University's Institute for Health and Care Research (EMGO+) and Neuroscience Campus Amsterdam (NCA); the European Community's Seventh Framework Program (FP7/2007-2013), ENGAGE (HEALTH-F4-2007-201413); and the European Science Council (ERC Advanced, 230374), Rutgers University Cell and DNA Repository (NIMH U24 MH068457-06), the Avera Institute, Sioux Falls, South Dakota (USA), and the National Institutes of Health (NIH, R01D0042157-01A, MH081802, Grand Opportunity grants 1RC2 MH089951). Part of the genotyping and analyses were funded by the Genetic Association Information Network (GAIN) of the Foundation for the National Institutes of Health. Computing was supported by BiG Grid, the Dutch e-Science Grid, which is financially supported by NWO.

FINRISK 1997 has been mainly funded by the budgetary funds of the National Institute for Health and Welfare. Important additional funding has been obtained from the Academy of Finland, Finnish Foundation for Cardiovascular Research and other domestic foundations. The NMR metabolomics determinations were funded by a grant from the Academy of Finland (#139635 to V.S.).

DILGOM 2007 baseline survey was funded by the Academy of Finland (grant # 136895 and 263836). V.S. was

supported by the Finnish Foundation for Cardiovascular Research.

SHIP is part of the Community Medicine Research Net of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research (grants no. 01ZZ9603, 01ZZ0103, and 01ZZ0403), the Ministry of Cultural Affairs as well as the Social Ministry of the Federal State of Mecklenburg-West Pomerania, and the network “Greifswald Approach to Individualized Medicine (GANI_MED)” funded by the Federal Ministry of Education and Research (grant 03IS2061A).

FHS: This work was supported by the dedication of the Framingham Heart Study participants. This work received support from the National Heart, Lung, and Blood Institute’s Framingham Heart Study (contracts no. N01-HC-25195 and HHSN268201500001I) and grants from the National Institute of Neurological Disorders and Stroke (NS17950 and UH2 NS100605), the National Institute on Aging (AG008122, R01 AG054076, R01 AG049607, R01 AG033193, U01 AG049505, and U01 AG052409) and the National Institute of Diabetes and Digestive and Kidney Diseases (R01-DK081572).

Estonian Biobank was funded by the European Union through the European Regional Development Fund in the framework of the Centre of Excellence for Genomics and Translational Medicine (GENTRANMED, Project No. 2014-2020.4.01.15-0012), ePerMed-EU 2020 grant no. 692145 and Estonian Research Council grant IUT20-60 and PUT1665.

AgeCoDe: We want to thank both all participating patients and their general practitioners for their good collaboration. We also thank all additional members of the AgeCoDe Study Group. This publication is part of the German Research Network on Dementia (KND) and the German Research Network on Degenerative Dementia (KNDD) and was funded by the German Federal Ministry of Education and Research (grants KND: 01GI0102, 01GI0420, 01GI0422, 01GI0423, 01GI0429, 01GI0431, 01GI0433, and 01GI0434; grants KNDD: 01GI0710, 01GI0711, 01GI0712, 01GI0713, 01GI0714, 01GI0715, 01GI0716, and 01ET1006B). The German Federal Ministry of Education and Research had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript. Analyses were also funded by the German Federal Ministry of Education and Research (BMBF 01EA1410A) within the project “Diet-Body-Brain: from epidemiology to evidence-based communication”.

VUmc Amsterdam Dementia Cohort: Research of the VUmc Alzheimer Center is part of the neurodegeneration research program of the Neuroscience Campus Amsterdam. The VUmc Alzheimer Center is supported by Stichting Alzheimer Nederland and Stichting VUmc fonds. The clinical database structure was developed with funding from Stichting Dioraphte. Metabolomics

measurements were funded by Biobanking and Biomolecular Resources Research Infrastructure (BBMRI)-NL (184.021.007). F.A.d.L. is appointed at the NWO-FCB project NUDAD (project number 057-14-004). C.E.T. serves on the advisory board of Fujirebio and Roche received research consumables from Euroimmun, IBL, Fujirebio, Invitrogen, and Meso Scale Discovery and performed contract research for IBL, Shire, Boehringer, Roche, and Probiobrug and received grants from the European Commission, the Dutch Research Council (ZonMW), Association of Frontotemporal Dementia/Alzheimer’s Drug Discovery Foundation, ISAO, and the Alzheimer’s Drug Discovery Foundation.

Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jalz.2017.11.012>.

RESEARCH IN CONTEXT

1. Systematic review: Cognitive function is an important indicator of brain health and a predictor of dementia. Metabolomics could provide valuable new insights into the determinants of cognitive function, but, to date, studies of blood metabolite measures and cognitive function are limited in size and findings are rarely replicated.
2. Interpretation: We undertook a large study on the associations of circulating metabolites with general cognitive ability and found a profile of 15 metabolites to be consistently associated with general cognitive ability, independently of high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, glucose, and *APOE* genotypes. Six of these metabolites were also associated with risk of dementia. The metabolites in the profile were associated with lifestyle factors.
3. Future directions: Future studies should examine the molecular mechanisms underlying the observed associations between metabolites, cognitive function, and dementia, whether metabolites can be used as readouts for preventive or therapeutic interventions, and whether selective interventions targeting metabolites would prevent dementia.

References

- [1] Alzheimer’s Association. 2015 Alzheimer’s disease facts and figures. *Alzheimers Dement* 2015;11:332–84.

- [2] Snyder HM, Corriveau RA, Craft S, Faber JE, Greenberg SM, Knopman D, et al. Vascular contributions to cognitive impairment and dementia including Alzheimer's disease. *Alzheimers Dement* 2015;11:710-7.
- [3] Solomon A, Kareholt I, Ngandu T, Winblad B, Nissinen A, Tuomilehto J, et al. Serum cholesterol changes after midlife and late-life cognition: twenty-one-year follow-up study. *Neurology* 2007;68:751-6.
- [4] Crichton GE, Elias MF, Davey A, Sullivan KJ, Robbins MA. Higher HDL cholesterol is associated with better cognitive function: the Maine-Syracuse study. *J Int Neuropsychol Soc* 2014;20:961-70.
- [5] Corley J, Starr JM, Deary IJ. Serum cholesterol and cognitive functions: the Lothian Birth Cohort 1936. *Int Psychogeriatr* 2015; 27:439-53.
- [6] Satizabal CL, Beiser AS, Chouraki V, Chene G, Dufouil C, Seshadri S. Incidence of dementia over three decades in the Framingham Heart Study. *N Engl J Med* 2016;374:523-32.
- [7] Rocca WA, Petersen RC, Knopman DS, Hebert LE, Evans DA, Hall KS, et al. Trends in the incidence and prevalence of Alzheimer's disease, dementia, and cognitive impairment in the United States. *Alzheimers Dement* 2011;7:80-93.
- [8] Schrijvers EM, Verhaaren BF, Koudstaal PJ, Hofman A, Ikram MA, Breteler MM. Is dementia incidence declining?: Trends in dementia incidence since 1990 in the Rotterdam Study. *Neurology* 2012; 78:1456-63.
- [9] Langa KM, Larson EB, Crimmins EM, Faul JD, Levine DA, Kabeto MU, et al. A comparison of the prevalence of dementia in the United States in 2000 and 2012. *JAMA Intern Med* 2017;177:51-8.
- [10] Henriksen K, O'Bryant SE, Hampel H, Trojanowski JQ, Montine TJ, Jeromin A, et al. The future of blood-based biomarkers for Alzheimer's disease. *Alzheimers Dement* 2014;10:115-31.
- [11] Hye A, Riddoch-Contreras J, Baird AL, Ashton NJ, Bazenet C, Leung R, et al. Plasma proteins predict conversion to dementia from prodromal disease. *Alzheimers Dement* 2014;10:799-807e2.
- [12] Muenchhoff J, Poljak A, Song F, Raftery M, Brodaty H, Duncan M, et al. Plasma protein profiling of mild cognitive impairment and Alzheimer's disease across two independent cohorts. *J Alzheimers Dis* 2015;43:1355-73.
- [13] Song F, Poljak A, Crawford J, Kochan NA, Wen W, Cameron B, et al. Plasma apolipoprotein levels are associated with cognitive status and decline in a community cohort of older individuals. *PLoS One* 2012; 7:e34078.
- [14] Proitsi P, Kim M, Whitley L, Simmons A, Sattlecker M, Velayudhan L, et al. Association of blood lipids with Alzheimer's disease: A comprehensive lipidomics analysis. *Alzheimers Dement* 2016;13:140-51.
- [15] Li D, Misialek JR, Boerwinkle E, Gottesman RF, Sharrett AR, Mosley TH, et al. Plasma phospholipids and prevalence of mild cognitive impairment and/or dementia in the ARIC Neurocognitive Study (ARIC-NCS). *Alzheimers Dement (Amst)* 2016;3:73-82.
- [16] Mapstone M, Cheema AK, Fiandaca MS, Zhong X, Mhyre TR, MacArthur LH, et al. Plasma phospholipids identify antecedent memory impairment in older adults. *Nat Med* 2014;20:415-8.
- [17] Fiandaca MS, Zhong X, Cheema AK, Orquiza MH, Chidambaram S, Tan MT, et al. Plasma 24-metabolite panel predicts preclinical transition to clinical stages of Alzheimer's disease. *Front Neurol* 2015; 6:237.
- [18] Casanova R, Varma S, Simpson B, Kim M, An Y, Saldana S, et al. Blood metabolite markers of preclinical Alzheimer's disease in two longitudinally followed cohorts of older individuals. *Alzheimers Dement* 2016;12:815-22.
- [19] Collins FS, Tabak LA. Policy: NIH plans to enhance reproducibility. *Nature* 2014;505:612-3.
- [20] Marmot MG, Smith GD, Stansfeld S, Patel C, North F, Head J, et al. Health inequalities among British civil-servants - the Whitehall-II study. *Lancet* 1991;337:1387-93.
- [21] van der Flier WM, Pijnenburg YA, Prins N, Lemstra AW, Bouwman FH, Teunissen CE, et al. Optimizing patient care and research: the Amsterdam Dementia Cohort. *J Alzheimers Dis* 2014; 41:313-27.
- [22] Davies G, Armstrong N, Bis JC, Bressler J, Chouraki V, Giddaluru S, et al. Genetic contributions to variation in general cognitive function: a meta-analysis of genome-wide association studies in the CHARGE consortium (N=53949). *Mol Psychiatry* 2015;20:183-92.
- [23] Johnson W, Nijenhuis J, Bouchard TJJ. Still just 1 g: Consistent results from five test batteries. *Intelligence* 2008;36:81-95.
- [24] Sabia S, Gueguen A, Marmot MG, Shipley MJ, Ankril J, Singh-Manoux A. Does cognition predict mortality in midlife? Results from the Whitehall II cohort study. *Neurobiol Aging* 2010; 31:688-95.
- [25] Spearman C. General intelligence, objectively determined and measured. *Am J Psychol* 1904;15:201-93.
- [26] Deary IJ, Pattie A, Starr JM. The stability of intelligence from age 11 to age 90 years: the Lothian birth cohort of 1921. *Psychol Sci* 2013; 24:2361-8.
- [27] Isaacs A, Sayed-Tabatabaei FA, Aulchenko YS, Zillikens MC, Sijbrands EJ, Schut AF, et al. Heritabilities, apolipoprotein E, and effects of inbreeding on plasma lipids in a genetically isolated population: the Erasmus Rucphen Family Study. *Eur J Epidemiol* 2007; 22:99-105.
- [28] de Bruijn RFAG, Bos MJ, Portegies MLP, Hofman A, Franco OH, Koudstaal PJ, et al. The potential for prevention of dementia across two decades: the prospective, population-based Rotterdam Study. *BMC Med* 2015;13:132.
- [29] Kalmijn S, Launer LJ, Ott A, Witteman JC, Hofman A, Breteler MM. Dietary fat intake and the risk of incident dementia in the Rotterdam Study. *Ann Neurol* 1997;42:776-82.
- [30] Hofman A, Brusselle GG, Darwish Murad S, van Duijn CM, Franco OH, Goedegebure A, et al. The Rotterdam Study: 2016 objectives and design update. *Eur J Epidemiol* 2015;30:661-708.
- [31] Sayed-Tabatabaei FA, van Rijn MJ, Schut AF, Aulchenko YS, Croes EA, Zillikens MC, et al. Heritability of the function and structure of the arterial wall: findings of the Erasmus Rucphen Family (ERF) study. *Stroke* 2005;36:2351-6.
- [32] van Gent CM, van der Voort HA, de Bruyn AM, Klein F. Cholesterol determinations. A comparative study of methods with special reference to enzymatic procedures. *Clin Chim Acta* 1977; 75:243-51.
- [33] Neeley WE. Simple automated determination of serum or plasma glucose by a hexokinase-glucose-6-phosphate dehydrogenase method. *Clin Chem* 1972;18:509-15.
- [34] Soininen P, Kangas AJ, Wurtz P, Tukiainen T, Tynkkynen T, Laatikainen R, et al. High-throughput serum NMR metabolomics for cost-effective holistic studies on systemic metabolism. *Analyst* 2009;134:1781-5.
- [35] Kettunen J, Demirkan A, Wurtz P, Draisma HH, Haller T, Rawal R, et al. Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. *Nat Commun* 2016; 7:11122.
- [36] Demirkan A, Henneman P, Verhoeven A, Dharuri H, Amin N, van Klinken JB, et al. Insight in genome-wide association of metabolite quantitative traits by exome sequence analyses. *PLoS Genet* 2015; 11:e1004835.
- [37] Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. *Heredity* 2005;95:221-7.
- [38] Wurtz P, Makinen VP, Soininen P, Kangas AJ, Tukiainen T, Kettunen J, et al. Metabolic signatures of insulin resistance in 7,098 young adults. *Diabetes* 2012;61:1372-80.
- [39] Wurtz P, Havulinna AS, Soininen P, Tynkkynen T, Prieto-Merino D, Tillin T, et al. Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. *Circulation* 2015; 131:774-85.
- [40] Shah AS, Tan L, Long JL, Davidson WS. Proteomic diversity of high density lipoproteins: our emerging understanding of its

- importance in lipid transport and beyond. *J Lipid Res* 2013; 54:2575–85.
- [41] Schwendeman A, Sviridov DO, Yuan WM, Guo YH, Morin EE, Yuan Y, et al. The effect of phospholipid composition of reconstituted HDL on its cholesterol efflux and anti-inflammatory properties. *J Lipid Res* 2015;56:1727–37.
- [42] Gordon SM, Deng J, Tomann AB, Shah AS, Lu LJ, Davidson WS. Multi-dimensional co-separation analysis reveals protein-protein interactions defining plasma lipoprotein subspecies. *Mol Cell Proteomics* 2013;12:3123–34.
- [43] Fellows K, Uher T, Browne RW, Weinstock-Guttman B, Horakova D, Posova H, et al. Protective associations of HDL with blood-brain barrier injury in multiple sclerosis patients. *J Lipid Res* 2015;56:2010–8.
- [44] Nguyen LN, Ma DL, Shui GH, Wong PY, Cazenave-Gassiot A, Zhang XD, et al. Mfsd2a is a transporter for the essential omega-3 fatty acid docosahexaenoic acid. *Nature* 2014;509:503–6.
- [45] Guemez-Gamboa A, Nguyen LN, Yang HB, Zaki MS, Kara M, Ben-Omran T, et al. Inactivating mutations in MFSD2A, required for omega-3 fatty acid transport in brain, cause a lethal microcephaly syndrome. *Nat Genet* 2015;47:809–13.
- [46] Cederholm T, Salem N, Palmblad J. Omega-3 fatty acids in the prevention of cognitive decline in humans. *Adv Nutr* 2013;4:672–6.
- [47] Morris MC, Brockman J, Schneider JA, Wang Y, Bennett DA, Tangney CC, et al. Association of seafood consumption, brain mercury level, and APOE epsilon4 Status with Brain neuropathology in older adults. *JAMA* 2016;315:489–97.
- [48] Huang TL. Omega-3 fatty acids, cognitive decline, and Alzheimer's disease: a critical review and evaluation of the literature. *J Alzheimers Dis* 2010;21:673–90.
- [49] Zhou Y, Danbolt NC. Glutamate as a neurotransmitter in the healthy brain. *J Neural Transm (Vienna)* 2014;121:799–817.
- [50] Nakanishi S. Molecular diversity of glutamate receptors and implications for brain function. *Science* 1992;258:597–603.
- [51] Fischer K, Kettunen J, Wurtz P, Haller T, Havulinna AS, Kangas AJ, et al. Biomarker profiling by nuclear magnetic resonance spectroscopy for the prediction of all-cause mortality: an observational study of 17,345 persons. *PLoS Med* 2014;11:e1001606.
- [52] Israili ZH, Dayton PG. Human alpha-1-glycoprotein and its interactions with drugs. *Drug Metab Rev* 2001;33:161–235.
- [53] Huang ZQ, Ung T. Effect of alpha-1-acid glycoprotein binding on pharmacokinetics and pharmacodynamics. *Curr Drug Metab* 2013; 14:226–38.
- [54] Harley J, Roberts R, Joyce P, Mulder R, Luty S, Frampton C, et al. Orosomucoid influences the response to antidepressants in major depressive disorder. *J Psychopharmacol* 2010;24:531–5.
- [55] Filosto M, Alberici A, Tessa A, Padovani A, Santorelli FM. Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome in adulthood: a rare recognizable condition. *Neurol Sci* 2013; 34:1699–701.
- [56] Ben-Zvi A, Lacoste B, Kur E, Andreone BJ, Mayshar Y, Yan H, et al. Mfsd2a is critical for the formation and function of the blood-brain barrier. *Nature* 2014;509:507–11.
- [57] Svennerholm L. Distribution and fatty acid composition of phosphoglycerides in normal human brain. *J Lipid Res* 1968;9:570–9.
- [58] Soderberg M, Edlund C, Kristensson K, Dallner G. Fatty acid composition of brain phospholipids in aging and in Alzheimer's disease. *Lipids* 1991;26:421–5.
- [59] Vitali C, Wellington CL, Calabresi L. HDL and cholesterol handling in the brain. *Cardiovasc Res* 2014;103:405–13.
- [60] Prasad MR, Lovell MA, Yatin M, Dhillon H, Markesbery WR. Regional membrane phospholipid alterations in Alzheimer's disease. *Neurochem Res* 1998;23:81–8.
- [61] van Wijk N, Slot RER, Duits FH, Strik M, Biesheuvel E, Sijben JWC, et al. Nutrients required for phospholipid synthesis are lower in blood and cerebrospinal fluid in mild cognitive impairment and Alzheimer's disease dementia. *Alzheimers Dement (Amst)* 2017;8:139–46.
- [62] Li H, Gordon SM, Zhu X, Deng J, Swertfeger DK, Davidson WS, et al. Network-based analysis on orthogonal separation of human plasma uncovers distinct high density lipoprotein complexes. *J Proteome Res* 2015;14:3082–94.
- [63] Sebastiao AM, Colino-Oliveira M, Assaife-Lopes N, Dias RB, Ribeiro JA. Lipid rafts, synaptic transmission and plasticity: impact in age-related neurodegenerative diseases. *Neuropharmacology* 2013;64:97–107.
- [64] Sonnino S, Aureli M, Grassi S, Mauri L, Prioni S, Prinetti A. Lipid rafts in neurodegeneration and neuroprotection. *Mol Neurobiol* 2014;50:130–48.
- [65] Singh-Manoux A, Shipley MJ, Bell JA, Canonico M, Elbaz A, Kivimaki M. Association between inflammatory biomarkers and all-cause, cardiovascular and cancer-related mortality. *CMAJ* 2016; 189:E384–90.
- [66] Guerrero-Berroa E, Ravona-Springer R, Heymann A, Schmeidler J, Levy A, Leroith D, et al. Haptoglobin genotype modulates the relationships of glycaemic control with cognitive function in elderly individuals with type 2 diabetes. *Diabetologia* 2015;58:736–44.
- [67] Fonteh AN, Harrington RJ, Tsai A, Liao P, Harrington MG. Free amino acid and dipeptide changes in the body fluids from Alzheimer's disease subjects. *Amino Acids* 2007;32:213–24.
- [68] Conneely KN, Boehnke M. Meta-analysis of genetic association studies and adjustment for multiple testing of correlated SNPs and traits. *Genet Epidemiol* 2010;34:739–46.
- [69] Johnson WM, McGue M, Lacono WG. Disruptive behavior and school grades: genetic and environmental relations in 11-year-olds. *J Educ Psychol* 2005;97:391–405.
- [70] Deary IJ, Johnson W. Intelligence and education: causal perceptions drive analytic processes and therefore conclusions. *Int J Epidemiol* 2010;39:1362–9.
- [71] Baltes PB, Reinert G. Cohort effects in cognitive development in children as revealed by cross-sectional sequences. *Dev Psychol* 1969;1:169–77.
- [72] Schmidt WHO. Socio-economic status, schooling, intelligence, and scholastic progress in a community in which education is not yet compulsory. *Paedagogica Europa* 1967;2:275–86.
- [73] Zheng J, Erzurumluoglu AM, Elsworth BL, Kemp JP, Howe L, Haycock PC, et al. LD Hub: a centralized database and web interface to perform LD score regression that maximizes the potential of summary level GWAS data for SNP heritability and genetic correlation analysis. *Bioinformatics* 2016;33:272–9.
- [74] Okbay A, Beauchamp JP, Fontana MA, Lee JJ, Pers TH, Rietveld CA, et al. Genome-wide association study identifies 74 loci associated with educational attainment. *Nature* 2016;533:539–42.
- [75] Johnson W, McGue M, Iacono WG. Genetic and environmental influences on academic achievement trajectories during adolescence. *Dev Psychol* 2006;42:514–32.