ELSEVIER

Alzheimer's & Dementia ■ (2018) 1-11

Alzheimer's & Dementia

Featured Article

Association of branched-chain amino acids and other circulating metabolites with risk of incident dementia and Alzheimer's disease: A prospective study in eight cohorts

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A.J.K., P.W., and P.S. are shareholders and report employment relation for Nightingale Health Ltd, a company offering NMR-based metabolite profiling. The other authors have no conflicts of interest.

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https://doi.org/10.1016/j.jalz.2018.01.003

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Abstract

Introduction: Metabolite, lipid, and lipoprotein lipid profiling can provide novel insights into mechanisms underlying incident dementia and Alzheimer's disease.

Methods: We studied eight prospective cohorts with 22,623 participants profiled by nuclear magnetic resonance or mass spectrometry metabolomics. Four cohorts were used for discovery with replication undertaken in the other four to avoid false positives. For metabolites that survived replication, combined association results are presented.

Results: Over 246,698 person-years, 995 and 745 cases of incident dementia and Alzheimer's disease were detected, respectively. Three branched-chain amino acids (isoleucine, leucine, and valine), creatinine and two very low density lipoprotein (VLDL)-specific lipoprotein lipid subclasses were associated with lower dementia risk. One high density lipoprotein (HDL; the concentration of cholesterol esters relative to total lipids in large HDL) and one VLDL (total cholesterol to total lipids ratio in very large VLDL) lipoprotein lipid subclass was associated with increased dementia risk. Branched-chain amino acids were also associated with decreased Alzheimer's disease risk and the concentration of cholesterol esters relative to total lipids in large HDL with increased Alzheimer's disease risk.

Discussion: Further studies can clarify whether these molecules play a causal role in dementia pathogenesis or are merely markers of early pathology.

Keywords: Dementia; Alzheimer's disease; Metabolomics; Biomarkers; Amino acids

1. Introduction

Dementia, including Alzheimer's disease (AD), is a major public health problem with devastating physical, financial, and social consequences for patients, their caregivers, families, and society. Worldwide the cost of AD care in 2010 was $604 billion or 1% of the global gross domestic product [1]. However, despite over two decades of research on animal models and clinical trials, we still have no effective prevention or disease-modifying therapy for late-onset clinical dementia and AD. Dementia is increasingly recognized as a heterogeneous syndrome that would be best addressed with a multipronged approach to prevention and treatment, analogous to the multipronged and individually tailored use of statins, antihypertensives, antiplatelet agents, and vasodilators in persons with coronary artery disease. Identifying novel biology could suggest new circulating biomarkers for risk prediction and drug targets. Agnostic approaches such as genome wide genetic analyses have identified new biological pathways and molecules mediating microglial inflammation (*TREM2*) and endocytosis (*BIN1*, *PICALM*) as having a previously unsuspected key role in AD pathophysiology [2,3].

Blood metabolomics is an attractive tool for agnostic exploration of disease pathways for several reasons. Metabolites are small molecules that reflect the interplay of genetic and environmental factors, readily cross the blood-brain barrier and their levels are modifiable through dietary or pharmacological interventions. This recognition has spurred interest in using metabolomics as a tool to understand AD. For example, longitudinal studies in mouse models of AD have implicated perturbed polyamine metabolism, disturbances in essential amino acids, branched-chain amino acids (BCAA), and in the neurotransmitter serotonin along with imbalances in phospholipid and acylcarnitine homeostasis in both the brain and the blood [4]. Human studies in cerebrospinal fluid and plasma have to date only compared AD cases to controls in cross-sectional settings or attempted to identify markers predicting conversion from mild cognitive impairment (MCI) to clinical dementia [5–9]. However, in persons with MCI or dementia, it is not possible to determine if the observed metabolite changes are causal or secondary to disease-related processes. There has only been one prior study of preclinical AD that failed to detect any consistently reproducible signal [10].

We conducted a prospective study relating blood metabolites, lipid, and lipoprotein lipids quantified by nuclear magnetic resonance (NMR) or mass spectrometry (MS) metabolomics to risk of incident dementia and AD in eight longitudinal studies with a total of 22,623 participants free of dementia at baseline: the FINRISK 1997 study, the Dietary, Lifestyle and Genetic determinants of Obesity and Metabolic Syndrome (DILGOM) study, the Whitehall II Study, the Estonian biobank study (EGCUT), the Health 2000, the Framingham Heart Study (FHS), the Rotterdam study (RS), and the Erasmus Ruchen Family (ERF) study. The first

four cohorts were used in discovery analyses, and the remaining four were used for replication. For metabolites taken to replication, we present overall association results combined across all eight samples by meta-analysis.

2. Methods

2.1. Cohorts

Eight prospective cohort studies were examined. Discovery cohorts were The National FINRISK Study 1997 (FINRISK 1997), DILGOM, Whitehall II, and the Estonian biobank study (EGCUT). Replication cohorts were the RS, ERF study, Health 2000 study, and the FHS. More detailed descriptions of each study are provided in the Supplementary Material (Supplements–Methods–Surveys).

Altogether, 22,623 participants were included in this study. The sample size and baseline characteristics of each study are presented in Supplementary Tables 1 and 2. All participants who at baseline examination had a history of doctor-diagnosed prevalent dementia, including AD, stroke, or other neurological disease affecting cognitive function, were excluded. No cognitive performance screening was conducted at baseline. Patients under 40 years of age were also excluded from all studies except ERF. All metabolite measurements were made from stored samples drawn at baseline of each cohort and no time-dependent covariates were used.

Dementia identification in FINRISK 1997, DILGOM, and Health 2000 cohorts was performed in the same manner, using country-wide, electronic health care registers: Causes of death Register, Hospital Discharge Register, and National Social Insurance Institution's Drug Reimbursement Register. In the EGCUT study cohort, participants were linked to the Estonian Health Insurance database containing detailed information on all contacts with health care services and prescriptions, and Estonian Causes of Death Registry, whereas prevalent disease information was additionally retrieved from recruitment questionnaires. In the Whitehall II study, participants were linked to electronic health records for dementia ascertainment using three databases: the national hospital episode statistics database, the Mental Health Services Data Set, and the mortality register. In the ERF survey, we used register data from general practitioner's databases (9 to 14 years after baseline visit). RS participants were screened for dementia at baseline and at follow-up examinations using a three-step protocol. Screen-positive participants subsequently underwent a more detailed examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly [11]. In addition, the total cohort was continuously monitored for dementia through computerized linkage of the study database with digitized medical records from general practitioners [11]. In the FHS, we screened participants at each examination, and between visits, for possible cognitive decline through a number of mechanisms, including an administration of the Folstein Mini-Mental Status Examination [12], participant and physician referrals, annual health status updates and review of medical records, and persons "flagged" as having possible cognitive decline underwent a more detailed neuropsychological and neurological evaluation. All cases were reviewed by a panel comprising at least one behavioral neurologist and one neuropsychologist. Details of end point detection are presented in the Supplementary Material (Supplements–Methods–Surveys). The cohort-specific dementia and AD detection methods are described in Supplementary Table 3.

2.2. Metabolomics analyses

A serum NMR metabolomics platform (Nightingale Health Ltd, Helsinki, Finland) was used to quantify 228 circulating metabolites, lipid or lipoprotein lipid measures in seven of the eight cohorts [13]. All tested metabolites are listed in Supplementary Table 4. This high-throughput metabolomics platform provides simultaneous quantification of routine lipids, lipid concentrations of 14 lipoprotein subclasses, and major subfractions, and further abundant fatty acids, amino acids, ketone bodies, and gluconeogenesis-related metabolites in absolute concentration units. The measured variables include 148 primary measures quantified in absolute concentrations as well as selected ratios, primarily related to fatty acids and lipoprotein composition. The NMR platform has been applied extensively in epidemiological studies [14,15], and details of the experimentation have been described elsewhere [13,16]. NMR analysis was used in all cohorts except FHS. In the FHS cohort, liquid chromatography-tandem mass spectrometry (LC-MS) has been used. LC-MS data were acquired using either an AB SCIEX 4000 QTRAP triple quadrupole mass spectrometer (positively charged polar compounds and lipids) or an AB SCIEX 5500 QTRAP triple quadrupole mass spectrometer (negatively charged polar compounds). Detailed protocols for the quantification of metabolites in the FHS have been previously published [17,18] and are described in Supplementary Material (Supplement–Methods–Metabolite analysis). A subset of 2638 serum samples from the FINRISK 1997 study were additionally profiled with LC-MS using the AbsoluteIDQ p180 Kit assay from Biocrates (Innsbruck, Austria). The correlations between circulating BCAA determined by NMR and LC-MS among the same individuals were fairly good as shown in Supplementary Fig. 1. The value of r^2 was 0.61 and 0.45 for valine and leucine, respectively.

2.3. Statistical analysis

In the discovery stage, we analyzed the associations of all metabolites, lipid, and lipoprotein lipid measures quantified by NMR metabolomics (n = 228) with incident dementia and AD. Two models were used: model 1 with age, sex, education grade, and number of apolipoprotein E (*APOE*) ε4 alleles as covariates and model 2 which additionally adjusted for systolic blood pressure, hypertension treatment, prevalent

diabetes, current smoking, and any prevalent cardiovascular disease (atrial fibrillation, coronary heart disease, heart failure, stroke, or peripheral artery disease). Because some of the NMR metabolites are highly correlated, we performed a principal component analysis to estimate the number of independent tests and corrected the *P*-values for multiple testing accordingly. The principal component analysis was conducted in the FINRISK 1997 cohort and 95% of the variation of NMR metabolites, lipids, and lipoprotein lipids was explained by 25 principal components, giving the corresponding *P*-value of .002 (0.05/25) as statistically significant (type I error correction). We used Cox proportional hazards regression model to test the metabolite associations. Time from the baseline examination to incident dementia, AD, death, or the end of the follow-up was used as the timescale, and age was used as a covariate in all models. Hazard ratios (HRs) and their 95% confidence intervals are presented per 1-SD of the rank inverse normal transformed concentration; 1-SD change in units is presented in Supplementary Table 4. Proportional hazard assumption was tested in two discovery cohorts (FINRISK 1997, DILGOM), and violations were observed for 14 metabolites (marked with asterisk in Supplementary Table 6). None of these metabolites or lipoprotein lipids were significant in the discovery analysis. All metabolites with *P*-value less than .002 in discovery meta-analyses were taken forward for testing in the replication cohorts. Because FHS did not use the NMR metabolomics platform, there were only a limited number of metabolites that could be replicated in the FHS cohort: valine, leucine, isoleucine, and creatinine. To examine the possible effect of selective mortality on our findings, we also conducted a sensitivity analysis using Fine-Gray subdistribution hazard modeling for the 10 discovered metabolites [19]. Death from any other cause than dementia was used as a competing risk in these models. The statistical analyses were carried out with R, version 3.2.3 or 3.3.1 using "survival," "cmprsk," and "meta" packages [20–22].

3. Results

The study included 22,623 subjects with 246,698 person-years of follow-up. In discovery cohorts, we observed altogether 329, 181, and 1435 cases of incident dementia, AD, and deaths from any cause, respectively. The EGCUT cohort did not record AD cases separately. The median follow-up times were 10.0, 7.9, 17.9, and 7.5 years (0.0, 0.1, 0.7, and 1.2 interquartile range) in FINRISK 1997, DILGOM, Whitehall II, and EGCUT cohorts, respectively (Supplementary Table 1).

In replication cohorts, we observed altogether 666, 466, and 1405 cases of incident dementia, AD, and deaths from any cause, respectively (Supplementary Table 2). The baseline characteristics for all cohorts are presented in Supplementary Tables 1 and 2. The classical risk factors for dementia, used as covariates in our models, produced the expected results (Supplementary Table 5). Covariates are described in Supplementary Material (Supplement–Methods–Covariates). As a positive control, it is worth noting that the number of *APOE* ε4 alleles was strongly associated with the risk of incident dementia (HR = 2.51, 95% confidence interval 2.00–3.16, $P < .001$, Supplementary Table 5)

3.1. Discovery and replication findings

In the discovery analyses (n = 15,161/329 study subjects/cases of incident dementia), altogether 10 metabolites or lipoprotein lipids were associated with incident dementia ($P < .002$) (Table 1) and none with incident AD. All the results of discovery analyses are presented in the Supplementary

Table 1
Metabolites and lipoprotein lipids associating statistically significantly ($P < .002$) with incident dementia in meta-analysis of discovery cohorts

Metabolite, lipid, lipoprotein lipid	FINRISK 1997, HR (95% CI)	DILGOM, HR (95% CI)	Whitehall, HR (95% CI)	EGCUT, HR (95% CI)	Fixed effect, HR (95% CI)	Random effect, HR (95% CI)	I2	*P*.fixed	*P*.random
Creatinine	0.92 (0.75; 1.12)	0.78 (0.58; 1.05)	0.64 (0.5; 0.82)	0.85 (0.59; 1.24)	0.8 (0.7; 0.91)	0.79 (0.67; 0.94)	0.383	<.001	.008
SFA-FA	1.13 (0.9; 1.4)	1.13 (0.84; 1.52)	1.31 (1.07; 1.6)	1.6 (1.17; 2.19)	1.26 (1.11; 1.42)	1.26 (1.09; 1.45)	0.227	<.001	.001
Isoleucine	0.9 (0.7; 1.16)	0.8 (0.59; 1.08)	0.72 (0.58; 0.9)	0.64 (0.42; 0.96)	0.78 (0.68; 0.89)	0.78 (0.68; 0.89)	0	<.001	<.001
Leucine	0.86 (0.66; 1.13)	0.72 (0.52; 1)	0.75 (0.6; 0.92)	0.54 (0.34; 0.84)	0.75 (0.65; 0.86)	0.74 (0.64; 0.86)	0.047	<.001	<.001
Valine	0.81 (0.62; 1.06)	0.64 (0.46; 0.89)	0.87 (0.72; 1.06)	0.59 (0.4; 0.88)	0.78 (0.69; 0.89)	0.76 (0.64; 0.9)	0.354	<.001	.002
L-HDL-CE-%	1.06 (0.87; 1.3)	1.25 (0.91; 1.71)	1.4 (1.13; 1.75)	1.39 (0.96; 2.02)	1.23 (1.09; 1.4)	1.24 (1.07; 1.44)	0.211	.001	.004
S-VLDL-C	1.01 (0.78; 1.3)	0.87 (0.62; 1.23)	0.72 (0.59; 0.87)	0.76 (0.53; 1.08)	0.81 (0.71; 0.92)	0.82 (0.69; 0.97)	0.355	.001	.023
XL-VLDL-C-%	0.94 (0.68; 1.29)	1.06 (0.72; 1.56)	1.28 (1.07; 1.53)	1.35 (1.07; 1.71)	1.22 (1.08; 1.38)	1.2 (1.03; 1.4)	0.259	<.002	.017
XL-VLDL-TG-%	1.03 (0.75; 1.42)	0.88 (0.59; 1.32)	0.78 (0.67; 0.91)	0.81 (0.62; 1.07)	0.82 (0.73; 0.93)	0.82 (0.73; 0.93)	0	.001	.001
Additional discoveries adjusted for model 1									
L-HDL-PL-%	0.94 (0.75; 1.17)	0.81 (0.58; 1.13)	0.76 (0.62; 0.93)	0.66 (0.44; 0.97)	0.81 (0.71; 0.92)	0.81 (0.71; 0.93)	0.067	<.002	.002

Abbreviations: DILGOM, Dietary, Lifestyle and Genetic determinants of Obesity and Metabolic Syndrome; EGCUT, Estonian biobank study; SFA-FA, Ratio of saturated fatty acids to total fatty acids; L-HDL-CE-%, Cholesterol esters to total lipids ratio in large HDL; S-VLDL-C, Total cholesterol in small VLDL; XL-VLDL-C-%, Total cholesterol to total lipids ratio in very large VLDL; XL-VLDL-TG-%, Triglycerides to total lipids ratio in very large VLDL.

NOTE. Hazard ratios (HRs) and 95% confidence intervals (CIs) are shown per one standard deviation (SD) of rank inverse normal transformed metabolite concentration. Adjusted for model 2* and the additional discoveries row adjusted for model 1†.

*Model 2 includes all of the above plus systolic blood pressure, hypertension treatment, prevalent diabetes, current smoking, and any prevalent cardiovascular disease (atrial fibrillation, coronary heart disease, heart failure, stroke, or peripheral artery disease) as covariates.

†Model 1 includes age, sex, education grade and number of *APOE* ε4 alleles as covariates.

Table 2
Replication results of the 10 preliminary significant metabolites associated with incident dementia in four separate cohorts

Metabolite, lipid, lipoprotein lipid	Health 2000, HR (95% CI)	Rotterdam, HR (95% CI)	ERF, HR (95% CI)	FHS, HR (95% CI)
Creatinine	1.19 (0.87; 1.63)	0.94 (0.84; 1.06)	0.82 (0.57; 1.18)	0.97 (0.78; 1.2)
SFA-FA	1.08 (0.78; 1.49)	0.94 (0.84; 1.04)	1.15 (0.61; 2.2)	NA
Isoleucine	0.87 (0.61; 1.25)	0.9 (0.8; 1.01)	0.66 (0.42; 1.03)	1.05 (0.84; 1.3)
Leucine	0.94 (0.69; 1.3)	0.84 (0.74; 0.94)	0.91 (0.6; 1.37)	0.94 (0.75; 1.19)
Valine	0.93 (0.68; 1.26)	0.86 (0.76; 0.96)	0.84 (0.55; 1.29)	0.96 (0.76; 1.19)
L-HDL-CE-%	0.99 (0.71; 1.38)	1.04 (0.94; 1.17)	1.08 (0.61; 1.91)	NA
L-HDL-PL-%	1.09 (0.8; 1.49)	1.04 (0.94; 1.16)	1.25 (0.74; 2.11)	NA
S-VLDL-C	0.89 (0.64; 1.23)	0.91 (0.82; 1.01)	1.08 (0.59; 1.97)	NA
XL-VLDL-C-%	0.87 (0.65; 1.16)	1.07 (0.95; 1.19)	0.67 (0.3; 1.49)	NA
XL-VLDL-TG-%	1.19 (0.87; 1.63)	0.94 (0.84; 1.06)	0.82 (0.57; 1.18)	0.97 (0.78; 1.2)

Abbreviations: ERF, Erasmus Ruchen Family; FHS, Framingham Heart Study; SFA-FA, Ratio of saturated fatty acids to total fatty acids; L-HDL-CE-%, Cholesterol esters to total lipids ratio in large HDL; S-VLDL-C, Total cholesterol in small VLDL; XL-VLDL-C-%, Total cholesterol to total lipids ratio in very large VLDL; XL-VLDL-TG-%, Triglycerides to total lipids ratio in very large VLDL; *APOE*, apolipoprotein E.

NOTE. Hazard ratio (HR) and 95% confidence intervals (CI) are shown per one standard deviation (SD) of rank inverse normal transformed metabolite concentration. Adjusted for model 2*.

*Model 2 includes age, sex, education grade, and number of *APOE* ε4 alleles plus systolic blood pressure, hypertension treatment, prevalent diabetes, current smoking, and any prevalent cardiovascular disease (atrial fibrillation, coronary heart disease, heart failure, stroke, or peripheral artery disease) as covariates.

Tables 6–9, for both models and both outcomes, dementia, and AD. The 10 metabolites and lipoprotein lipids were tested further in four replication cohorts (Health 2000, RS, ERF, and FHS), and these results are shown in Table 2 (dementia, model 2) and Supplementary Tables 10–12 (dementia model 1 and AD models 1–2). None of the statistically significant metabolite associations were driven by a single cohort and the results appeared to be consistent in all discovery and replication cohorts (Supplementary Figs. 2–3).

3.2. Meta-analysis of all cohorts

The results of the discovery and replication cohorts were combined in meta-analysis of all cohorts (n = 22,623/995 study subjects/cases of incident dementia). All three BCAAs (isoleucine, leucine, and valine) were inversely associated with incident dementia (model 2), (Fig. 1, Supplementary Tables 13 and 14). Also creatinine, total cholesterol in small VLDL (S-VLDL-C), and triglycerides to total lipids ratio in very large VLDL were inversely associated with incident dementia (Fig. 1, Supplementary Tables 13 and 14). The concentration of cholesterol esters relative to total lipids in large HDL (L-HDL-CE-%) and total cholesterol to total lipids ratio in very large VLDL were directly associated with incident dementia (Fig. 1) (Supplementary Tables 13 and 14). The HRs for AD were broadly similar but smaller numbers of incident AD cases compared to incident dementia diluted the statistical significance of the results (Fig. 1, Supplementary Tables 15 and 16). The correlations between the metabolites associated with incident dementia are presented in Supplementary Table 17. Of note are the moderately strong correlations between the BCAAs and S-VLDL-C (r = 0.67–0.55).

3.3. Sensitivity analyses

We carried out two sensitivity analyses. First, to examine the influence of body mass index (BMI) and cholesterol-lowering medication on incident dementia and AD risk. Second, to examine the possible effects of selective mortality, we conducted Fine-Gray subdistribution hazard analysis for the 10 discovered metabolites. These analyses are briefly described in the next two paragraphs.

The associations of BCAAs, L-HDL-CE-%, and S-VLDL-C with incident dementia remained similar after adjusting for BMI and cholesterol-lowering medication (Supplementary Table 18). However none of the discovered metabolites were associated with incident AD after the analyses were adjusted for BMI and cholesterol-lowering medication (Supplementary Tables 18 and 19).

Next, we hypothesized that the associations of metabolites with incident dementia or AD might be confounded by a competing risk of death because the elevated levels of, for example, BCAA have been associated with metabolic syndrome, diabetes, and cardiovascular events. In Fine-Gray subdistribution hazard analysis, creatinine and BCAAs remained significantly associated with lower, and L-HDL-CE-% with higher, dementia risk (Supplementary Table 20). S-VLVD-C was not associated with incident dementia in Fine-Gray analysis (Supplementary Table 20). Other HDL- or VLDL lipoprotein subclasses were not associated with dementia risk in Fine-Gray analysis. We did not observe any statistically significant associations between metabolites and incident AD in Fine-Gray analysis. This is not surprising because all Fine-Gray results were adjusted for model 2 and BMI.

In a meta-analysis of FINRISK 1997, DILGOM, and Health 2000 results, we observed no interactions between metabolites, lipids, or lipoprotein lipids, and *APOE* ε4 genotype or sex on the association ($P < .002$) with incident dementia or AD.

4. Discussion

Our study identified 10 metabolites or lipoprotein lipids associated with the risk for clinically incident dementia

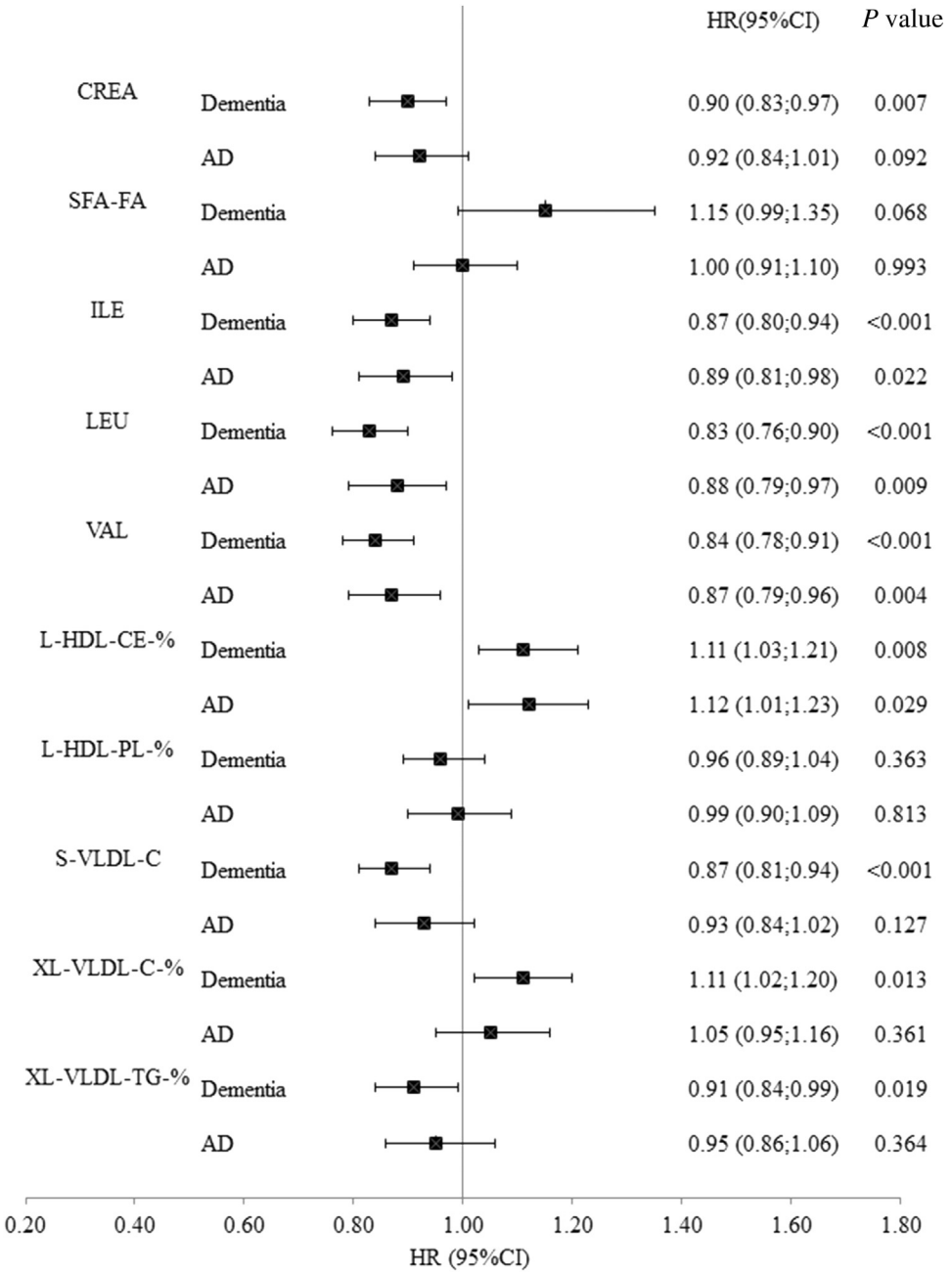

Fig. 1. A forest plot describing the meta-analysis results of all eight cohorts combined (n = 22,623/995, participants/incident dementia cases). Results are adjusted for model 2. Model 2 includes age, sex, education grade, number of *APOE* ε4 alleles, systolic blood pressure, hypertension treatment, prevalent diabetes, current smoking, and any prevalent cardiovascular disease (atrial fibrillation, coronary heart disease, heart failure, stroke, or peripheral artery disease) as covariates. HRs and 95% CIs are shown per one SD of rank inverse normal transformed metabolite concentration. Abbreviations: APOE, apolipoprotein E; CIs, confidence intervals; HRs, hazard ratios; SD, standard deviation; AD, Alzheimer's disease; CREA, creatinine; SFA-FA, Ratio of saturated fatty acids to total fatty acids; ILE, isoleucine; LEU, leucine; VAL, valine; L-HDL-CE-%, Cholesterol esters to total lipids ratio in large HDL; L-HDL-PL-%, phospholipids to total lipids ratio in large HDL; S-VLDL-C, Total cholesterol in small VLDL; XL-VLDL-C-%, Total cholesterol to total lipids ratio in very large VLDL; XL-VLDL-TG-%, Triglycerides to total lipids ratio in very large VLDL.

across four discovery cohorts. Lower levels of the BCAA such as valine were associated with an increased risk of both all dementia and of AD in this discovery cohort and in a combined meta-analysis with a replication sample. In addition, we observed inverse associations of creatinine, total cholesterol in S-VLDL-C, and triglycerides to total lipids ratio in very large VLDL with incident dementia, but not with AD in the discovery cohort alone. In meta-analysis, the concentration of L-HDL-CE-% was associated with an increased risk of AD.

We tested 228 metabolic measures quantified by serum NMR metabolomics in the discovery sample. We chose a conservative strategy of initial discovery followed by independent replication of a hard clinical end point, to minimize

the risk of reporting false-positive associations, a pitfall that has impacted earlier reports. This may have however reduced our ability to identify some true associations, for example, with docosahexaenoic acid which, in addition to earlier literature [23,24], was recently discovered to associate with higher general cognitive ability in a study based partly on the same cohorts as the present study (Sven J. van der Lee, personal communication, article submitted).

To our knowledge, the inverse association of BCAAs with clinical dementia has not been reported previously. In line with our results, Toledo et al. observed higher valine level to be associated with slower cognitive decline and lesser cerebral atrophy change in the "Alzheimer's Disease Neuroimaging Initiative" cohort [25].

Although there are biologically plausible explanations for such an association for what we describe, subsequently we wished to explore whether these findings could represent reverse causality or selective survival. Valine, leucine, and isoleucine are essential BCAAs, and circulating levels are largely determined by dietary intake. Thus, reduced levels of these essential amino acids might indicate subclinical nutritional deficiencies in persons with preclinical dementia and MCI [26]. Indeed, in later life, weight loss is known to be associated with a higher risk of dementia, and it has been associated with declining BCAA levels [27,28]. However, plasma albumin, which is used as a marker of nutrition, was not related to dementia risk and BCAA's HRs for incident dementia remained similar after adjusting for BMI. BCAAs are also associated with muscle mass [29], which is consistent with our observation of an inverse association of creatinine and dementia risk. Hence, it is possible that these metabolites are early markers of MCI, reduced physical activity, and muscle mass.

Competing risk of death in persons with elevated BCAA levels is not likely to explain the observed inverse associations with incident dementia because BCAAs remained associated with incident dementia in Fine-Gray subdistribution model. In a recent study, Pedersen et al demonstrated that gut microbiota are an independent source of BCAAs [30], but these have not been examined in relation to dementia risk although changes in gut microbiota have been associated with other neuropsychiatric diseases [31].

Lower cerebrospinal fluid valine has been recorded in persons with AD dementia compared to controls [32], although some studies have shown low valine in MCI and higher values in AD [33]. In a small cross-sectional study, no differences were seen in BCAA blood levels among healthy controls, patients with MCI, and those with AD [34], but an independent cross-sectional study identified valine, among other metabolites, as an indicator for disease progression from MCI to AD [33]. It is hypothesized that circulating BCAAs could have an important role in glutamate synthesis and could also buffer toxic levels of glutamate [35]. Glutamate is the most abundant excitatory neurotransmitter and binds to cell surface receptors like α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors and N-methyl-D-aspartate receptors [36]. Because N-methyl-D-aspartate-receptor hypofunction seems to be related to calcium ion dysregulation and impaired synaptic plasticity [37], it is possible that the association of reduced levels of BCAA to dementia and AD is mediated through this pathway [33]. Higher valine has also been associated with increased apoptosis in maple syrup urine disease, and lower valine in preclinical AD could be a compensatory phenomenon in response to activation of apoptotic pathways [38]. Nevertheless, it should be noted that once the models were adjusted for BMI and cholesterol-lowering medication, the associations between BCAAs and incident AD were no longer statistically significant. The robust association of these metabolites with all dementia and vanishing association with AD may reflect the smaller numbers of AD cases or be due to a stronger association with vascular dementia.

A VLDL-receptor polymorphism has been reported to associate with dementia risk, especially with mixed and vascular dementia [39]. We detected total VLDL cholesterol to be inversely associated with dementia risk as did Lara et al. [40] The association between VLDL cholesterol and cardiovascular events [14,41] is one possible explanation for our observation. In addition, we observed one VLDL lipoprotein subclass to decrease (triglycerides to total lipids ratio in very large VLDL) and one to increase (total cholesterol to total lipids ratio in very large VLDL) the dementia risk. To the best of our knowledge, no association between large HDL lipoprotein and dementia risk has been published previously. Cholesterol-lowering medication affects lipoprotein levels, and these associations, except L-HDL-CE-% and S-VLDL-C, with incident dementia were attenuated after adjusting for BMI and cholesterol-lowering medication. Association of S-VLDL-C with incident dementia can be largely explained by increased selective mortality. We believe that the associations between HDL lipoprotein and VLDL lipoprotein subclasses and dementia risk need more research because our findings and the previous studies are inconclusive.

We did not observe any robust associations of cholesterols, triglycerides, phospholipids, apolipoproteins, fatty acids, glycolysis-related metabolites, ketone bodies, fluid balance metabolites, or inflammation markers with incident dementia or AD. In previous studies, several specific phospholipids have been shown to associate with the risk of conversion of MCI to AD [6,9], but the metabolomics platform used here does not share the same phospholipids reported on in those earlier studies.

Our results also differ from prior studies which suggested an association of sphingomyelin and docosahexaenoic acid with dementia and AD [6,23,24,42]; these differences could be due to chance or differences in dietary patterns or genetic risk between the cohorts studied here and in early reports. Docosahexaenoic acid and eicosapentaenoic acid dietary supplements have been associated with better cognitive function [43,44], and lower odds of dementia and

AD (personal communication, Sven J. van der Lee) in some studies but two large meta-analyses did not show an association, suggesting that this area deserves further scrutiny including possible gene-environment interactions [45]. Our conservative statistical strategy and lower sensitivity to detect some associations may partly explain these differences between our study and previous studies as described above.

We did not observe any effect modification of the metabolites or lipoprotein lipids by *APOE* genotype or sex in their effect on dementia or AD risk. We did not observe any robust associations with absolute concentrations of lipoproteins and incident dementia or AD. This is in line with previous genetic studies where no causal effect of circulating levels of HDL cholesterol, serum total cholesterol, LDL cholesterol, or triglycerides on incident AD was seen, despite the genetic associations with the *APOE* and *ABCA7* loci [46,47]. Age, education level, *APOE* genotype, and prevalent diabetes were the only covariates associated with incident dementia in this study. We did not observe statistically significant association between incident dementia and smoking, systolic blood pressure, heart failure, or atrial fibrillation. This is not surprising because the analyses were not designed to investigate these associations, and survival bias likely affects these results.

The strengths of our study include the large samples and prospective population-based design with separate discovery and replication cohorts. Furthermore, all NMR metabolomics measurements were carried out in the same laboratory following the same protocol, and only one cohort used a different methodology. Limitations of the study include some differences between cohorts in the methods used to identify cases of incident dementia. Most cohorts relied on electronic health registers, which leads to virtually complete follow-up and high specificity but may have limited sensitivity [48]. The lower sensitivity potentially reduces the power to detect statistically significant associations but should not result in spurious associations. Moreover, the findings were replicated in prospective cohorts such as the RS and FHS that used more sensitive, surveillance-based outcome ascertainment. Some cohorts were unable to distinguish AD from other dementias, which led to a reduced statistical power for the AD analysis. Ethnic homogeneity of our largely Caucasian sample limits the generalizability of these results to other populations with different ethnic backgrounds. Also, the FHS used an MS platform, and therefore, only a limited set of metabolites were in common between the FHS and other cohorts. The main association we report here, of BCAA, was however directionally consistent in FHS and the other cohorts and the correlations between the BCAAs measured using MS and NMR were reasonably strong.

Several next steps can be considered to clarify our findings further. The earlier studies that have reported an association between BCAA and metabolic syndrome and diabetes risk, should be examined for availability of cognitive end points and possible confirmation of the present findings. Furthermore, a Mendelian randomization study on BCAA and other metabolites and incident dementia or AD should help with causal inferences. A Mendelian randomization study on BCAAs and risk of type 2 diabetes has been published suggesting that suitable single-nucleotide polymorphisms exist [49], although this work has been recently criticized for the possibility of misinterpretation due to pleiotropism [50]. If further evidence supporting causality is obtained, a clinical trial supplementing BCAA in diet could be considered but such a trial should be carefully designed and monitored also for diabetes and other metabolic outcomes. A wider angle is the rapidly increasing availability of metabolomics profiling. Researchers should consider establishing an international metabolomics consortium aiming at harmonizing measures and analysis protocols across metabolomics platforms and cohorts, so that data already collected can be meta-analyzed. This would also encourage metabolomics studies in other geographical areas and other ethnic groups, which are clearly needed. Another line of research that is worth pursuing in parallel to confirming the discovery findings is the prediction of dementia or AD risk. Even if not causal, these biomarkers may improve the prediction of incident dementia or AD over and above the currently used risk scores. This in turn could enable earlier starting and better targeting of medical and other treatments, which has the potential to slow down the cognitive decline.

In conclusion, our large prospective study identified lower BCAA levels to be associated with an increased risk of incident dementia, independent of other conventional risk factors. Moreover, creatinine, one HDL, and three VLDL lipoprotein subclasses were also associated with dementia risk, but these associations disappeared when adjusted for BMI and cholesterol-lowering medication. Further studies are needed to explore whether these metabolites play a role in the etiology and pathogenesis of dementia, or reflect reverse causation, that is, they are biomarkers for systemic or lifestyle changes in the preclinical stages of dementia. In either case, if corroborated in other studies, these biomarkers may help in early identification of persons at risk of dementia and initiation of preventive and treatment measures.

Acknowledgments

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 (no. 139635 to V.S.).

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

Health 2000 is funded by the National Institute for Health and Welfare (THL), the Finnish Centre for Pensions (ETK), The Social Insurance Institution of Finland (KELA), The Local Government Pensions Institution (KEVA), and other organizations listed on the website of the survey

(www.thl.fi/fi/tutkimus-ja-asiantuntijatyo/vaestotutkimukset/terveys-2000-2011/yhteistyokumppanit).

EGCUT was supported by European Union H2020 grants 692145, Est.RC grant IUT20-60 (A.M.) and PUT1665P (K.F.) EU Project no. 2014-2020.4.01.15-0012 (Gentransmed) and from the Estonian Ministry of Social Affairs.

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). 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 5th Framework Programme (no. QLG2-CT-2002-01254); FP7 project EUROHEADPAIN (no. 602633), the Internationale Stichting Alzheimer Onderzoek (ISAO); the Hersenstichting Nederland (HSN); and the JPND under the project PERADES (grant number 733051021, Defining Genetic, Polygenic and Environmental Risk for Alzheimer's Disease using multiple powerful cohorts, focused Epigenetics, and Stem cell metabolomics). Metabolomics measurements of ERF have been funded by Biobanking and Biomolecular Resources Research Infrastructure (BBMRI)–NL (184.021.007) and. A.D. is supported by a Veni grant (2015) from ZonMw. The ERF follow-up study is funded by CardioVasculair Onderzoek Nederland (CVON 2012-03). The authors are grateful to all study participants and their relatives, general practitioners, and neurologists for their contributions and P. Veraart for her help in genealogy, J. Vergeer for the supervision of the laboratory work, both Sven J. van der Lee 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: The metabolomics profiling for the Rotterdam Study 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). Funding was further provided by the Netherlands Organisation for Health Research and Development (ZonMW) as part of the Joint Programming for Neurological Disease (JPND) as part of the program PERADES (Defining Genetic, Polygenic and Environmental Risk for Alzheimer's Disease using multiple powerful cohorts, focused Epigenetics and Stem cell metabolomics - grant number 733051021). This study was also funded by 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); 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); 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 European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (project: ORACLE, grant agreement No. 678543); The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, the Netherlands Organization for Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the municipality of Rotterdam. The authors are grateful to the study participants, the staff from the Rotterdam Study, and the participating general practitioners and pharmacists. Generation and management of the Illumina exome chip v1.0 array data for the Rotterdam Study (RS-I) was executed by the Human Genotyping Facility of the Genetic Laboratory of the Department of Internal Medicine (http://www.glimdna.org/), Erasmus MC, Rotterdam, the Netherlands. Generation and management of GWAS genotype data for the Rotterdam Study (RS-I, RS-II, RS-III) was executed by the Human Genotyping Facility of the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, Rotterdam, the Netherlands. The GWAS data sets are supported by the Netherlands Organization of Scientific Research NWO Investments (175.010.2005.011, 911-03-012), the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), and the Netherlands Genomics Initiative (NGI)/Netherlands Organization for Scientific Research (NWO) Netherlands Consortium for Healthy Aging (NCHA), project 050-060-810. This study makes use of an extended data set of RS-II and RS-III samples based on Illumina Omni 2.5 and 5.0 GWAS genotype data. This data set was funded by the Genetic Laboratory of the Department of Internal Medicine, the Department of Forensic Molecular Biology, and the Department of Dermatology, Erasmus MC, Rotterdam, the Netherlands. The Whitehall II study: The UK Medical Research Council (K013351; G0902037), the British Heart Foundation, and the US National Institutes of Health (R01HL36310, R01AG013196) have supported collection of data in the Whitehall II study.

The Framingham Heart Study: This work was supported by the dedication of the Framingham Heart Study participants. This work received grants from the National Institute on Aging (R01 AG054076, AG016495, AG049505, AG049607, and AG033193), the National Institute of Neurological Disorders and Stroke (NS017950), and the National Institute of Diabetes and Digestive and Kidney Diseases (R01-DK-HL081572) and support from the National Heart, Lung, and Blood Institute's Framingham Heart Study (contracts no. N01-HC-25195 and HHSN268201500001I).

P.W. is supported by the Academy of Finland (294834) and the Novo Nordisk Foundation. The serum NMR metabolomics platform has been supported by the Sigrid Juselius Foundation and the Strategic Research Funding from the University of Oulu. M.A.K. works in a unit that is supported by the University of Bristol and UK Medical Research Council (MC_UU_1201/1).

The funding agencies 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 article; and decision to submit the article for publication. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Neurological Disorders and Stroke; the National Heart, Lung, and Blood Institute; the National Institute of Aging; the National Institute of Diabetes and Digestive and Kidney Diseases; or the National Institutes of Health. The information contained in this article does not necessarily reflect the position or the policy of the U.S. government, and no official endorsement should be inferred.

Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jalz.2018.01.003.

RESEARCH IN CONTEXT

1. Systematic review: Blood metabolites are small molecules that reflect the interplay between genetic and environmental factors, readily cross the blood-brain barrier, and have the potential to play a role in the development of dementia. Well-powered prospective studies on metabolome and incident dementia or Alzheimer's disease are limited at the moment.
2. Interpretation: We carried out a large prospective study on metabolite, lipid, and lipoprotein lipid associations with incident dementia and Alzheimer's disease in eight cohorts. Branched-chain amino acids isoleucine, leucine, and valine and cholesterol ester ratio in large HDL had robust associations with the risk of dementia and Alzheimer's disease. Selective mortality did not explain these associations.
3. Future directions: Future mechanistic studies should examine whether the identified metabolites or lipoprotein lipids play etiologic roles in the development of dementia or whether they are early biomarkers of mild cognitive decline and developing physical frailty.

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