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Association of amine biomarkers with incident dementia and Alzheimer's disease in the Framingham Study

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Abstract

INTRODUCTION—The identification of novel biomarkers associated with Alzheimer's disease (AD) could provide key biological insights and permit targeted preclinical prevention. We

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None

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investigated circulating metabolites associated with incident dementia and AD using metabolomics.

METHODS—Plasma levels of 217 metabolites were assessed in 2067 dementia-free Framingham Offspring Cohort participants (mean age=55.9±9.7 years; 52.4% women). We studied their associations with future dementia and AD risk in multivariate Cox models.

RESULTS—Ninety-three participants developed incident dementia (mean follow-up=15.6±5.2 years). Higher plasma anthranilic acid levels were associated with greater risk of dementia (HR=1.40; 95%CI=[1.15–1.70]; $p=8.08\times10^{-4}$). Glutamic acid (HR=1.38; 95%CI=[1.11–1.72]), taurine (HR=0.74; 95%CI=[0.60–0.92]) and hypoxanthine (HR=0.74; 95%CI=[0.60–0.92]) levels also showed suggestive associations with dementia risk.

DISCUSSION—We identified four biologically plausible, candidate plasma biomarkers for dementia. Association of anthranilic acid implicates the kynurenine pathway, which modulates glutamate excitotoxicity. The associations with hypoxanthine and taurine strengthen evidence that uric acid and taurine may be neuroprotective.

Keywords

Dementia; Alzheimer's disease; Cohort studies; Epidemiology; Plasma Biomarkers; Metabolomics; Anthranilic Acid; Kynurenines; Glutamic Acid; Taurine; Hypoxanthine; Uric Acid

Background

Alzheimer's disease (AD) is the most frequent form of dementia, and a growing public health burden. [1] However, to date, there is no effective preventive or curative intervention. One reason may be that AD has a long preclinical phase, starting over two decades before clinical onset, so that intervention after the onset of clinical dementia is too late to prevent further progression. [2,3] Therefore, the identification of novel AD biological pathways that could provide new drug targets and easily detectable circulating biomarkers predicting risk of dementia is an urgent priority. [4] Existing biomarkers have been developed based on the “amyloid cascade” hypothesis of AD in which the disease results from the aggregation of amyloid beta peptides in the brain, leading, through a cascade of events, to the hyperphosphorylation of tau proteins, axonal loss, neuronal death, and clinical onset. [5] Biomarkers derived from those peptides, when measured in the cerebrospinal fluid (CSF) or used as targets in Positron Emission Tomography (PET)-scans, are used to refine the diagnosis of AD. But the cost of PET-scans is still very high, and the acceptability of lumbar puncture is low in an asymptomatic population, thus limiting their use in AD risk prediction.

Outside the amyloid cascade, other biomarkers studied in the Framingham cohorts using a candidate approach have shown some significant associations with risk of AD and led to additional studies of their role in AD pathophysiology. [6–9] Given the expected complexity of AD and the relative success of agnostic genome-wide association studies, which suggested that additional biological pathways may modulate the risk of AD, [10,11] a broader selection of circulating biomarkers might further improve our understanding of AD biology and risk prediction. The metabolomics approach studies the products of cell and organismal metabolism. Assessing simultaneously the circulating levels of a large number of

metabolites allows the construction and comparison of metabolic profiles between pathological conditions and enhances the discovery of new biomarkers. Using those techniques in animal models and in human participants, in blood, CSF or postmortem brain, several previously unsuspected mechanisms have been implicated in the pathophysiology of AD, such as phospholipids and the tryptophan, purine and tyrosine pathways. [12–14] Nevertheless, the results have been inconsistent, likely due to differences in analytical platforms, choice of outcome measures and frequently, cross-sectional study designs that make it difficult to assess whether the observed changes preceded, or are consequent to the dementia process.

In this study, we used data from a prospective community-based cohort to perform an exploratory analysis of plasma metabolomics data and to identify individual circulating biomarkers and biological pathways associated with risk of developing dementia and AD.

Methods

1 Study Samples

The Framingham Heart Study (FHS) is an ongoing community-based prospective cohort study initiated in 1948 with the enrollment of 5209 women and men aged 28 to 74 years (Original Cohort). [15] In 1971, offspring of the Original Cohort and the spouses of these offspring (n=5124; age, 5–70 years; 3548 biological offspring and 1576 offspring spouses) were enrolled in the Framingham Offspring Cohort. [16] They have been examined every four to eight years since, nine times to date, for a core examination. [17] In addition, the Offspring cohort has been under ongoing surveillance for cognitive decline and dementia since the fifth examination (1991–1995, n=3799). A total of 2526 participants among those who attended this fifth examination had their plasma metabolome measured. We excluded participants with prevalent dementia (n=133), no follow-up (n=43), or missing values for selected covariates (education level, APOEε4 status and/or homocysteine; n=629) yielding a subsample of up to 2067 participants for longitudinal assessment of dementia and AD risks related to metabolite concentrations (see Supplementary Methods for the complete flowchart of the study). The study protocol was approved by the Institutional Review Board of the Boston University Medical Center, and all participants provided written informed consent.

2 Dementia and AD Assessment

Detailed procedures for dementia screening and assessment have been published previously. [18] We screened participants at each examination for possible cognitive decline using serial administrations of the Folstein Mini-Mental Status Examination (MMSE). [19] Any of the following participant scores resulted in an MMSE flag: an absolute score of <23 for all persons, a score <24 for persons with a high school education, a score <26 for college-educated persons; a decline of 3 points since the participant's previous exam; or a decline of 5 points from their personal best score. Participants could also be flagged through 45-minutes neuropsychological tests they underwent every 5 or 6 years since 1999. Finally, participants might also be “flagged” by self, family or physician expressed concern either spontaneous or at a health status update, a Framingham Heart Study ancillary study examination or a Framingham Heart Study baseline examination.

Persons flagged as having possible cognitive decline or otherwise being at risk for developing dementia underwent a more detailed neuropsychological and neurological evaluation and when required a structured family interview was administered to one or more family members and caregivers over the telephone. All persons were assigned a Clinical Dementia Rating [20] scale score. We then determined whether each person fulfilled criteria for a diagnosis of dementia, the probable date of onset, and type of dementia at a consensus review conducted by a panel comprising at least one behavioral neurologist and one neuropsychologist. Participants with dementia met criteria outlined in the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders criteria. [21] Participants with AD met National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association criteria for definite, probable, or possible AD. [22] For the present analyses, data for incident dementia obtained till December 2013 were used.

3 Assessment of Metabolites

Detailed protocols for the quantification of metabolites in the FHS have been previously published. [23–25] Briefly, the metabolome was assessed in FHS Offspring on plasma samples collected following an overnight fast and stored at -80°C (see Supplementary Methods). Plasma metabolites were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS). 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). Polar, positively charged metabolites were separated using hydrophobic interaction liquid chromatography (HILIC) and analyzed using multiple reaction monitoring (MRM) in the positive ion mode. Polar, negatively charged compounds, including central and polar phosphorylated metabolites, were separated using a Luna NH2 column (150×2 mm, Luna NH2, Phenomenex) and analyzed using MRM in the negative ion mode. Lipids were separated on a Prosphere C4 HPLC column and underwent full scan MS analysis in the positive ion mode. MultiQuant software (Version 1.2, AB SCIEX) was used for automated peak integration and manual review of data quality prior to statistical analysis. Quality control and normalization of metabolite levels are detailed in Supplementary Methods.

4 Assessment of Covariates

Educational achievement was defined as a three class variable (no high-school degree, high-school degree only, or at least a college degree). The presence or absence of APOE ϵ 4 alleles was determined by isoelectric focusing of the plasma and confirmed by DNA genotyping. Plasma total homocysteine levels were determined by high-performance liquid chromatography with fluorometric detection, as previously described. [26] We additionally adjusted for vascular risk factors that are included in the widely used Framingham Stroke Risk Profile: systolic blood pressure recorded as the mean of two physician-recorded measurements in millimeters of mercury, use of anti-hypertensive therapy, diabetes mellitus (defined as a fasting blood glucose concentration >7 mmol/L, a previous diagnosis of diabetes mellitus, or the use of a hypoglycemic agent or insulin), current smoking status, presence or absence of atrial fibrillation and previous cardiovascular disease (a diagnosis of

coronary heart disease, congestive heart failure, stroke or peripheral vascular disease). In addition to anti-diabetic and anti-hypertensive medications, we also considered cholesterol-lowering drugs, including reported use of resins, niacin, fibrates and statins classes in sensitivity analyses.

5 Statistical Analysis

We log-transformed metabolite levels to account for the skewness of their distributions, and standardized them to reflect risk associated with a difference of one standard deviation in statistical analyses. For persons with missing covariate data at the 5th Offspring examination, these data were imputed based on recorded levels at the previous or subsequent examinations attended by the participant, if available.

For each metabolite, longitudinal associations of metabolite levels with risk of incident dementia and AD were estimated in Cox regression models using hazard ratios with their 95% confidence intervals (95% CI). Two successive adjustment strategies were used, first for factors known to predict risk of dementia in the FHS sample: age, sex, batch, APOEε4 status, education level and log-transformed total homocysteine (model 1), and then with additional adjustment for the cardiovascular variables listed earlier (model 2). For the metabolites we identified, we also performed sensitivity analyses by excluding participants who were less than 60 years old at the start of follow-up (hence too young to be at risk for clinical dementia), by adjusting for concomitant medication use (cholesterol-lowering and antidepressant drugs) and by adding an interaction term with sex. A previous analysis of the correlation structure of available metabolites [27] yielded 37 independent clusters of metabolites that explained 98% of the total variance and were used to determine a Bonferroni-corrected p-value threshold of 1.35×10^{-3} (0.05/37). We performed an enrichment analysis using Metaboanalyst 3.0 and a list of 14 metabolites that were associated with incident risk of dementia with a p-value below 0.05 (see Supplementary Table 1). [28] Analyses and plots were performed using SAS version 9.2 (SAS Institute, Cary, NC, USA) and R version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

A total of 2067 participants (mean age 55.3 ± 9.5 , 52% women) were followed over an average period of 15.8 ± 5.2 years (32276.93 person-years) during which 93 developed incident dementia, including 68 with incident AD (Table 1). After quality control, 217 metabolites were available for analysis: 54 amines and related metabolites in up to 2067 participants, 59 “central” metabolites (organic acids and related metabolites) in up to 1690 participants and 104 lipids in up to 1692 participants (see Supplementary Table 1 for a complete listing). Participants excluded from analyses were on average younger and were more likely to have a history of diabetes or cardiovascular disease (see Supplementary Tables 2–4).

P-values from tests for association between risk of incident dementia and each of the 217 metabolites available are summarized in the Figure. Considering a p-value threshold of 1.35×10^{-3} in a model adjusted for age, sex, education, APOEε4 status and total homocysteine levels, we observed a significant association between plasma anthranilic acid

levels and risk of incident dementia, with a risk increased by 40% for an increase of one standard deviation (Hazard Ratio (HR)=1.40; 95% Confidence Interval (CI)=[1.15 – 1.70]; p-value=8.08x10⁻⁴; see Table 2 and Figure 2). Using a more liberal p-value threshold of 10⁻², suggestive associations with incident dementia were found for three additional metabolites: glutamic acid (HR=1.38; 95%CI=[1.11 – 1.72]; p-value=3.80x10⁻³), taurine (HR=0.74; 95%CI=[0.60 – 0.92]; p-value=6.91 x10⁻³) and hypoxanthine (HR=0.74; 95%CI=[0.60 – 0.92]; p-value=6.93x10⁻³) (see Table 2 and Supplementary Figure 1). These four metabolites showed little correlation between each other (Supplementary Figure 2), with the exception of anthranilic acid and glutamic acid, which were positively correlated (spearman r=0.56). Additional adjustment for vascular risk factors did not substantially modify the observed effects (Table 2), neither did additional adjustment for cholesterol-lowering drugs, antidepressant drugs and exclusion of participants aged below 60 (Supplementary Table 5). Finally we did not find significant interaction between those four metabolites and sex (Supplementary Table 6).

When focusing on risk of incident AD alone, only glutamic acid maintained similar levels of p-values and hazard ratios (Table 2). The associations between anthranilic acid and hypoxanthine remained stable in terms of HR estimation, but were no longer significant. Finally, the association of taurine levels was no longer apparent.

As anthranilic acid is part of the kynurenine pathway (KP), we also investigated other available metabolites from this pathway, namely tryptophan, 3-hydroxy-anthranilic acid, kynurenic acid, kynurenine, quinolinic acid and xanthurenic acid. Spearman correlation coefficients showed inverse correlation between anthranilic acid and hydroxy-anthranilic acid and positive correlation between quinolinic acid and kynurenine (Supplementary Figure 3). Apart from anthranilic acid, no other association between metabolites from the KP and incident dementia of AD reached nominal significance (Table 3). As expected from the correlation structure, 3-hydroxy-anthranilic acid showed a protective, albeit nonsignificant association with risk of dementia and AD (HR=0.76; 95%CI=[0.57 – 1.03]; p-value=7.42x10⁻² and HR=0.83; 95%CI=[0.59 – 1.16]; p-value=2.81x10⁻¹, respectively; Table 3). When studying ratios of KP metabolites, we observed suggestive levels of association between kynurenine over tryptophan (KYN/TRP) ratio with risk of incident dementia (HR= 1.43; 95%CI=[1.03 – 1.99]; p-value=3.10x10⁻²).

In enrichment analyses, we observed suggestive significance for pathways mainly involving glutamic acid and glutamine, e.g. "glutamate metabolism" and "ammonia recycling" (Supplementary Table 7). Similarly, we obtained suggestive significance for disease pathways such as "heart failure" and "schizophrenia" (Supplementary Table 8).

Discussion

In this study, we assessed the associations of 217 plasma metabolites with risk of incident dementia and AD in the Framingham Offspring Cohort. After correction for multiple testing, we observed a significant association between higher plasma anthranilic acid levels and an increased risk of incident dementia. Three other metabolites, glutamic acid, taurine and

hypoxanthine, were associated at a suggestive level of significance. When focusing on AD, the associations were robust except for plasma taurine levels.

Several metabolomics studies have already been reported in the field of AD and dementia. [12–14] Given the relatively high cost of metabolite assessment, the sample sizes are usually low, below a hundred, and the design is often cross-sectional, which precludes study of metabolite profiles in a preclinical context. Another issue is the small overlap in available metabolites between the various analytical platforms, preventing rapid replication of results. For example, lipidomics have been a very fruitful approach in the field of AD and several combination of lipids with very high discrimination of AD cases and controls have been reported. [13,14] In this study, we did not find any significant association between lipids levels and risk of incident dementia or AD. Compared to the “amines” group of metabolites, lipids were analyzed in a smaller sample which could have limited statistical power. Moreover, using agnostic approaches imposed using multiple test correction that might have been conservative and lead us to discard otherwise interesting lipids signals.

Plasma anthranilic acid levels were associated with increased risk of incident dementia and AD. Conversely, plasma 3-hydroxy-anthranilic acid levels were associated with a decreased risk of incident dementia and AD, although this association was not significant. A change in the ratio of these two amines has been related to several neurological and psychiatric conditions, such as stroke, Huntington's disease, and depression. [29] Reports of associations with dementia and AD are scarce: a study of plasma kynurenines in blood did not show significant difference in anthranilic acid level between of 34 AD cases and 18 controls. [30]

Although they are not fully characterized, several mechanisms related to neurodegeneration could explain how both metabolites modulate risk of dementia and AD. First, 3-hydroxy-anthranilic acid and anthranilic acid could have pro- or anti-oxidant activity involving chelation of iron, with potential toxic consequences on certain cell types. [31] Secondly, both anthranilic acid and 3-hydroxy-anthranilic acid are able to inhibit the activity of the 3-hydroxy-anthranilic acid oxydase (3HAO) which in turn, inhibits the production of quinolinic and picolinic acid, both known to exert toxic effects on neurons. [29] Finally, 3-hydroxy-anthranilic acid levels have been involved in immunosuppression either through direct modulation of T cells activity or inhibition of T cell activity by dendritic cells. [29] Unlike 3-hydroxy-anthranilic acid, anthranilic acid can cross the blood brain barrier and could exert its effect directly on neurons. [32]

Anthranilic acid and 3-hydroxy-anthranilic acid both belong to the KP. This pathway represents the main degradation path of tryptophan, an essential amino acid which is also the precursor of serotonin. Two separate branches lead to the production of kynurenic acid in astrocytes, and quinolinic acid in microglia, the latter being a precursor of NAD⁺. [33] In our study, we did not find significant association between plasma levels of other metabolites from the KP and risk of incident dementia or AD. Other metabolomics studies have reported alteration of tryptophan metabolism, either in plasma [34] or in the CSF of patients with AD or mild cognitive impairment (MCI). [12,35] One study reported lower levels of tryptophan and kynurenic acid and higher levels of quinolinic acid in the plasma of 34 AD cases compared to 18 controls. [30] Interestingly, we observed suggestive association of

KYN/TRP ratio, a marker of KP activation, and risk of incident dementia. This observation is coherent with previous reports showing higher KYN/TRP ratio in the plasma and serum of AD cases compared to controls [30,36,37] and in the CSF of MCI patients compared to controls. [12]

In addition to mechanisms related to oxidative stress and regulation of the immune response, metabolites in this pathway are able to modulate excitotoxicity through glutamate release and glutamate reuptake at the synapse, quinolinic acid being neurotoxic and kynurenic acid neuroprotective. [38] The KP can be activated in response to various pro-inflammatory factors, including A β 1–42, [39] and this process has been observed in the brain of AD patients at the level of amyloid plaques. [40] Inflammation is now considered an important and early event in AD pathophysiology. [41] We could therefore hypothesize that the KP is first activated in microglial cells and astrocytes to modulate neuroinflammation. In the long run, metabolites from the KP, especially quinolinic acid, could participate to neurodegeneration through glutamate excitotoxicity. [38] Inhibition of key enzymes of the KP resulted in amelioration of neurodegeneration in mice and fly models of AD. [42,43] Thus, metabolites from the KP could provide a range of promising therapeutic targets. [44]

Except for the liver, the KP is initiated and completed at different locations. Tryptophan, kynurenine, 3-hydroxy-kynurenine, and anthranilic acid are able to cross the blood–brain barrier whereas 3-hydroxy-anthranilic acid and quinolinic acid are not. [32] It is therefore plausible that the associations we observed does not only reflect a specific effect on neurodegeneration. Metabolites from the KP have been associated with other diseases that could subsequently modulate risk of dementia, such as obesity, [45] acute coronary events [46] and stroke [47] although the effect size we observed was not reduced by adjustment for vascular risk factors, suggesting the observed effect was not chiefly due to vascular injury. Finally, as tryptophan metabolism is conserved across species and tightly regulated, metabolites from the KP could be involved more broadly in the aging processes. [48]

Apart from anthranilic acid, three other metabolites showed suggestive associations with risk of dementia. Glutamic acid is the major excitatory neurotransmitter in the brain, is implicated in the pathology of AD, and its release and recapture can be modulated by metabolites from the KP. CSF Glutamic acid levels have been associated with AD status [13,14] although reports have been contradictory. Finally, the glutamatergic system can be targeted by memantine in the management of clinical AD.

Taurine, the most abundant sulfur-containing amino-acid, can cross the blood brain barrier and could be protective against glutamate toxicity. Increased dietary taurine has been postulated to improve cognitive function. [49] The fact that a protective effect was only seen with all cause dementia and not AD in our sample suggests that taurine acts through vascular rather than AD neurodegenerative pathways to clinical dementia. This is plausible as taurine is involved in blood pressure regulation and low dietary taurine is associated with hypertension in epidemiological studies. [50] Two recent studies have shown that taurine could improve cerebral blood flow, mitochondrial function and reduce hypercoagulability in an animal model of traumatic brain injury, [51] and lower blood pressure in a small clinical trial, [52]

Hypoxanthine is a purine derivative, a product of DNA metabolism following apoptosis and cell lysis. It can then be processed further into uric acid or “rescued” by the hypoxanthine-guanine phosphoribosyltransferase enzyme and recycled into DNA synthesis. Hypoxanthine levels might therefore reflect the activity of the cell cycle or cell death or implicate the uric acid cycle. The association we observed between higher plasma levels of hypoxanthine and a lower risk of incident AD is consistent with other reports of a potential neuroprotective effect of uric acid. Two meta-analyses have suggested that circulating levels of uric acid might be lower in AD cases compared to controls. [53,54] Two other studies reported associations between a diagnosis of gout and lower risk of incident AD [55] and between high levels of serum uric acid and lower risk of all incident dementia. [56] The purine pathway as a whole has been implicated in AD in one prior CSF metabolomics study. [12]

Our study has several strengths. It uses a prospective cohort design wherein metabolites were measured in cognitively normal adults, so the associations observed are unlikely a consequence of clinical dementia. Dementia and AD have been assessed following rigorous standardized protocols, as were the covariates used in the analyses. All metabolites were assessed in the same, well-established laboratory, following robust and reproducible protocols and up-to-date technologies such as liquid chromatography and mass spectrometry.

We also acknowledge some limitations. Our choice of using a Bonferroni correction to set the significance threshold may have been too conservative and hence we may have missed some real associations of metabolites with risk of dementia. This is also true since despite our large sample size, the number of incident dementia cases was relatively small. We were not able to find other prospective cohort studies where plasma anthranilic acid levels are available to replicate our results but our conservative statistical threshold and the evidence of biological plausibility suggest our results are real. We studied plasma rather than CSF or brain metabolite concentrations and hence can only draw indirect inferences about their relevance to pathological processes in the brain. Further, as these metabolites are involved in highly conserved reactions, their levels might reflect broader aging or vascular changes. Although the measure of the metabolome in our middle-aged population is relevant with regard to the expected long preclinical phase of AD, the optimal timing of metabolome assessment for dementia and AD risk prediction has yet to be determined. Finally, as the risk of AD is low before the age of 60 and we decided to include all available samples to maximize statistical power, we might have reduced the comparability between the participants that developed incident dementia and those who did not, especially with regards to age, body composition and co-morbidities. Nevertheless, limiting our analyses to participants aged 60 and older had little impact on effect sizes.

In the Framingham Heart Study, elevated plasma anthranilic acid levels were associated with a higher risk of incident dementia and AD, implicating the tryptophan-kynurenine pathway, which has been linked to glutamate excitotoxicity in the pathogenesis of dementia. Our data also support prior reports that the amino-acid taurine and the purine hypoxanthine, levels of the latter paralleling uric acid levels, are protective against the risk of clinical dementia. Whether these results reflect a specific involvement of these metabolites in the

pathophysiology of AD or act through vascular or other aging-related pathways has yet to be determined.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We used metabolomics to identify plasma biomarkers of incident dementia and AD
- Higher levels of anthranilic acid were associated with greater risk of incident dementia
- Anthranilic acid, along other kynurenine pathway metabolites, could modulate glutamate excitotoxicity
- Higher glutamic acid and lower taurine and hypoxanthine (precursor of uric acid) levels were also associated with increased risk of subsequent dementia
- These data support evidence from animal studies that uric acid and taurine may be neuroprotective

Research in Context

Systematic review

We sought to identify novel biology and biomarkers associated with incident dementia and AD using an agnostic approach. Higher levels of plasma anthranilic acid are associated with a greater risk of incident dementia in 2067 participants from the Framingham Offspring Study. We also found suggestive associations for glutamic acid, taurine and hypoxanthine.

Interpretation

These findings strengthen the previously suspected involvement of the kynurenine pathway in glutamatergic excitotoxicity and neurodegeneration and the protective roles of taurine and the uric acid pathway.

Future directions

Further human studies are needed to confirm these associations, although they are biologically plausible based on animal studies. At this time, we could not find other prospective cohorts with available data on both plasma anthranilic acid and dementia risk. An international collaboration is being initiated to facilitate standardized platforms, measurements and meta-analyses.

Figure 1.

Manhattan Plot of the Associations with Risk of Incident Dementia across the Three Sets of Metabolites in the Framingham Offspring Cohort.

NOTES. P-values are represented on the y-axis after $-\log_{10}$ transformation; thus the higher a dot is, the lower the p-value. Metabolites are represented on the x-axis, grouped by categories. The plain line represents the significance threshold of 1.35×10^{-3} calculated in this study. The dotted line represents a suggestive threshold of 10^{-2} .

Figure 2.
Kaplan-Meier curves of survival without dementia according to quartiles of anthranilic acid

Table 1

Main characteristics of the study population at exam 5 (1991–1995)

	N	Dementia-free at the end of follow-up N = 1974	Incident dementia event N = 93	Combined N = 2067	P-value
Follow-up Time years	2067	13.3 18.2 19.7	7.2 11.3 14.2	13.0 18.0 19.7	< 0.001 ¹
Age years	2067	55.3±9.5	67.8±6.1	55.9±9.7	< 0.001 ¹
Male	2067	48% (939)	48% (45)	48% (984)	0.877 ²
APOEε4 status	2067	22% (433)	41% (38)	23% (471)	< 0.001 ²
Total Homocysteine Level μmol/L	2067	7.7 9.5 11.7	8.0 9.7 12.3	7.7 9.5 11.8	0.249 ¹
Education Level	2067				< 0.001 ²
No High school degree		6% (112)	15% (14)	6% (126)	
High school degree		59% (1163)	60% (56)	59% (1219)	
College graduate		35% (699)	25% (23)	35% (722)	
Systolic Blood Pressure mmHg	2067	126.1±18.6	135.0±22.2	126.5±18.9	< 0.001 ¹
Anti-hypertensive Medication	2067	19% (371)	40% (37)	20% (408)	< 0.001 ²
Diabetes	2067	6% (123)	11% (10)	6% (133)	0.082 ²
Current Smoking	2067	19% (376)	8% (7)	19% (383)	0.005 ²
History of CVD	2067	8% (166)	18% (17)	9% (183)	0.001 ²
History of Atrial Fibrillation	2067	2% (42)	2% (2)	2% (44)	0.988 ²
Left Ventricular Hypertrophy	2056	1% (13)	1% (1)	1% (14)	0.62 ²

a b c represent the lower quartile a, the median b, and the upper quartile c for continuous variables. x±s represents mean ± 1 SD. N is the number of non-missing values. Numbers after percents are frequencies. Tests used: 1Wilcoxon test; 2Pearson test

Table 2

Association of the top four metabolites with incident dementia and Alzheimer's disease

Metabolites	N. Total	Dementia			Alzheimer's Disease				
		N. Events	HR	95% CI	P-value	N. Events	HR	95% CI	P-value
Anthranilic Acid									
<i>model 1</i>	2067	93	1.40	1.15 – 1.70	8.08 x 10 ⁻⁰⁴	68	1.35	1.07 – 1.70	1.26 x 10 ⁻⁰²
<i>model 2</i>	2056	91	1.38	1.12 – 1.69	2.47 x 10 ⁻⁰³	66	1.32	1.03 – 1.69	2.98 x 10 ⁻⁰²
Glutamic Acid									
<i>model 1</i>	2067	93	1.38	1.11 – 1.72	3.80 x 10 ⁻⁰³	68	1.45	1.11 – 1.88	5.86 x 10 ⁻⁰³
<i>model 2</i>	2056	91	1.33	1.06 – 1.66	1.34 x 10 ⁻⁰²	66	1.37	1.04 – 1.79	2.33 x 10 ⁻⁰²
Taurine									
<i>model 1</i>	2067	93	0.74	0.60 – 0.92	6.91 x 10 ⁻⁰³	68	0.91	0.70 – 1.18	4.81 x 10 ⁻⁰¹
<i>model 2</i>	2056	91	0.74	0.59 – 0.92	7.22 x 10 ⁻⁰³	66	0.90	0.69 – 1.18	4.52 x 10 ⁻⁰¹
Hypoxanthine									
<i>model 1</i>	1625	69	0.74	0.60 – 0.92	6.93 x 10 ⁻⁰³	53	0.74	0.57 – 0.95	1.72 x 10 ⁻⁰²
<i>model 2</i>	1618	67	0.74	0.59 – 0.91	5.58 x 10 ⁻⁰³	51	0.73	0.56 – 0.94	1.42 x 10 ⁻⁰²

ABBREVIATIONS: HR: Hazard Ratio; CI: Confidence Interval NOTES: HR are for one SD increase in a given metabolite level; model 1 is adjusted for age, sex, education, APOEε4 status and total homocysteine levels; model 2 has additional adjustment for systolic blood pressure, antihypertensive medication status, diabetes status, current smoking status, history of cardiovascular disease, history of atrial fibrillation and left ventricular hypertrophy

Table 3
Association of the available metabolites from the kynurenine pathway with incident dementia and Alzheimer's disease.

Metabolites	N.Total	Dementia			Alzheimer's Disease				
		N. Events	HR	95% CI	P-value	N. Events	HR	95% CI	P-value
Tryptophan									
model 1	2067	93	0.92	0.74 – 1.15	4.59 x 10 ⁻⁰¹	68	0.94	0.72 – 1.22	6.36 x 10 ⁻⁰¹
model 2	2056	91	0.91	0.73 – 1.14	4.03 x 10 ⁻⁰¹	66	0.94	0.72 – 1.23	6.54 x 10 ⁻⁰¹
Kynurenine									
model 1	1690	73	1.08	0.83 – 1.42	5.67 x 10 ⁻⁰¹	55	1.15	0.84 – 1.58	3.75 x 10 ⁻⁰¹
model 2	1682	71	1.03	0.78 – 1.37	8.28 x 10 ⁻⁰¹	53	1.12	0.80 – 1.55	5.10 x 10 ⁻⁰¹
Kynurenic Acid									
model 1	2067	93	1.06	0.84 – 1.32	6.37 x 10 ⁻⁰¹	68	1.21	0.92 – 1.59	1.75 x 10 ⁻⁰¹
model 2	2056	91	1.04	0.82 – 1.31	7.45 x 10 ⁻⁰¹	66	1.21	0.91 – 1.60	1.90 x 10 ⁻⁰¹
Anthranilic Acid									
model 1	2067	93	1.40	1.15 – 1.70	8.08 x 10 ⁻⁰⁴	68	1.35	1.07 – 1.70	1.26 x 10 ⁻⁰²
model 2	2056	91	1.38	1.12 – 1.69	2.47 x 10 ⁻⁰³	66	1.32	1.03 – 1.69	2.98 x 10 ⁻⁰²
3-Hydroxy-anthranilic Acid									
model 1	1255	49	0.76	0.57 – 1.03	7.42 x 10 ⁻⁰²	38	0.83	0.59 – 1.16	2.81 x 10 ⁻⁰¹
model 2	1249	48	0.76	0.56 – 1.03	8.09 x 10 ⁻⁰²	37	0.82	0.58 – 1.17	2.82 x 10 ⁻⁰¹
Xanthurenic Acid									
model 1	1688	73	0.93	0.73 – 1.18	5.42 x 10 ⁻⁰¹	55	0.93	0.71 – 1.21	5.83 x 10 ⁻⁰¹
model 2	1680	71	0.93	0.73 – 1.19	5.72 x 10 ⁻⁰¹	53	0.95	0.72 – 1.24	6.88 x 10 ⁻⁰¹
Quinolinic Acid									
model 1	1690	73	1.12	0.87 – 1.43	3.74 x 10 ⁻⁰¹	55	1.04	0.78 – 1.40	7.66 x 10 ⁻⁰¹
model 2	1682	71	1.07	0.83 – 1.38	6.02 x 10 ⁻⁰¹	53	1.01	0.74 – 1.36	9.74 x 10 ⁻⁰¹
Kynurenine / Tryptophan Ratio									
model 1	1690	73	1.43	1.03 – 1.99	3.10 x 10 ⁻⁰²	55	1.35	0.86 – 2.13	1.95 x 10 ⁻⁰¹
model 2	1682	71	1.45	1.03 – 2.03	3.11 x 10 ⁻⁰²	53	1.35	0.85 – 2.16	2.03 x 10 ⁻⁰¹
3-Hydroxy-anthranilic Acid / Anthranilic Acid Ratio									
model 1	1255	49	0.99	0.80 – 1.21	8.91 x 10 ⁻⁰¹	38	0.97	0.79 – 1.19	7.60 x 10 ⁻⁰¹

Metabolites	N.Total	Dementia				Alzheimer's Disease			
		N. Events	HR	95% CI	P-value	N. Events	HR	95% CI	P-value
<i>model 2</i>	1249	48	0.96	0.76 – 1.21	7.53 x 10 ⁻⁰¹	37	0.95	0.77 – 1.18	6.43 x 10 ⁻⁰¹
Quinolinic Acid / Kynurenic Acid Ratio									
<i>model 1</i>	1690	73	0.91	0.76 – 1.10	3.44 x 10 ⁻⁰¹	55	0.94	0.69 – 1.28	6.99 x 10 ⁻⁰¹
<i>model 2</i>	1682	71	0.91	0.76 – 1.10	3.24 x 10 ⁻⁰¹	53	0.94	0.69 – 1.27	6.72 x 10 ⁻⁰¹

ABBREVIATIONS: HR: Hazard Ratio; CI: Confidence Interval NOTES: HR are for one SD increase in a given metabolite level; model 1 is adjusted for age, sex, education, APOEε4 status and total homocysteine levels; model 2 has additional adjustment for systolic blood pressure, antihypertensive medication status, diabetes status, current smoking status, history of cardiovascular disease, history of atrial fibrillation and left ventricular hypertrophy