Phosphorus metabolism in the brain of cognitively normal midlife individuals at risk for Alzheimer’s disease

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Abstract

\textbf{Background:} Neurometabolic abnormalities and amyloid-beta plaque deposition are important early pathophysiologic changes in Alzheimer’s disease (AD). This study investigated the relationship between high-energy phosphorus-containing metabolites, glucose uptake, and amyloid plaque using phosphorus magnetic resonance spectroscopy ($^{31}$P-MRS) and positron emission tomography (PET).

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Additional information

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ynirp.2022.100121.
Methods: We measured $^{31}$P-MRS, fluorodeoxyglucose (FDG)-PET, and Pittsburgh Compound B (PiB)-PET in a cohort of 20 cognitively normal middle-aged adults at risk for AD. We assessed $^{31}$P-MRS reliability by scanning a separate cohort of 13 healthy volunteers twice each. We calculated the coefficient-of-variation (CV) of metabolite ratios phosphocreatine-to-adenosine triphosphate (PCr/$\alpha$-ATP), inorganic phosphate (Pi)-to-$\alpha$-ATP, and phosphomonoesters-to-phosphodiesters (PME/PDE), and pH in pre-defined brain regions. We performed linear regression analysis to determine the relationship between $^{31}$P measurements and tracer uptake, and Dunn’s multiple comparison tests to investigate regional differences in phosphorus metabolism. Finally, we performed linear regression analysis on $^{31}$P-MRS measurements in both cohorts to investigate the relationship of phosphorus metabolism with age.

Results: Most regional $^{31}$P metabolite ratio and pH inter- and intra-day CVs were well below 10%. There was an inverse relationship between FDG-SUV levels and metabolite ratios PCr/$\alpha$-ATP, Pi/$\alpha$-ATP, and PME/PDE in several brain regions in the AD risk group. There were also several regional differences among $^{31}$P metabolites and pH in the AD risk group including elevated PCr/$\alpha$-ATP, depressed PME/PDE, and elevated pH in the temporal cortices. Increased PCr/$\alpha$-ATP throughout the brain was associated with aging.

Conclusions: Phosphorus spectroscopy in the brain can be performed with high repeatability. Phosphorus metabolism varies with region and age, and is related to glucose uptake in adults at risk for AD. Phosphorus spectroscopy may be a valuable approach to study early changes in brain energetics in high-risk populations.

Keywords
Phosphorus magnetic resonance spectroscopy; Brain energy metabolism; Positron emission tomography

1. Introduction

Neurometabolic abnormalities and amyloid-beta plaque deposition have been identified as important early pathophysiologic changes in Alzheimer’s disease (AD) (Atlante et al., 2017; Demetrius et al., 2014; Jack et al., 2016; Scheltens et al., 2021). Plaque deposition has been primarily studied by measuring Pittsburgh Compound B (PiB) uptake with positron emission tomography (PET), with signature patterns of retention in the frontal, parietal, temporal, and occipital cortex, and the striatum (Klunk et al., 2004). Metabolic impairment has been primarily studied by measuring glucose uptake with $[18F]$-fluorodeoxyglucose (FDG) PET, with characteristically reduced glucose metabolism in AD-vulnerable brain regions (Chetelat et al., 2003; Nestor et al., 2003). While FDG-PET is sensitive to cerebral cellular glucose uptake and incorporation after the first phosphorylation, phosphorus MR spectroscopy ($^{31}$P-MRS) can probe high-energy phosphates, such as adenosine triphosphate (ATP), phosphocreatine (PCr), and inorganic phosphate (Pi), as well as metabolites of phospholipid membranes (phosphomonoesters (PME), and phosphodiesters (PDE)), alterations of which are associated with impairment in energy storage and membrane synthesis or breakdown (Forester et al., 2010; Pettegrew et al., 1987).
Historically, $^{31}$P-MRS of the brain has been carried out on 1.5 T MRI systems that necessitated unlocalized measurements (Longo et al., 1993; Murphy et al., 1993; Bottomley et al., 1992), and with radiofrequency surface coils, which limited coverage and introduced inhomogeneous spin excitation (Smith et al., 1995; Pettegrew et al., 1994). Modern systems operating at 3 T or higher with volume or phased array coils have allowed for improved $^{31}$P-MRS resolution, which provides the opportunity to explore regional and anatomically localized changes in the brain (Brown et al., 1995; Luyten et al., 1989; Bachert-Baumann et al., 1990; Mathur-De Vre et al., 1990; Lagemaat et al., 2016; Bottomley and Hardy, 1992; Lei et al., 2003a; Lei et al., 2003b; Parasoglo et al., 2013; Stoll et al., 2016; Rodgers et al., 2014; Hattingen et al., 2009a; Hattingen et al., 2011; Hattingen et al., 2009b; Das et al., 2021).

We carried out $^{31}$P-MRS measurements on a 3 T system with a dual-tuned $^3$H/$^{31}$P multichannel coil array, a setup that is known to improve sensitivity over a volume coil (Brown et al., 2016a, 2016b; Avdievich et al., 2020; Valkovic et al., 2017; Avdievich and Hetherington, 2007), to investigate: 1) $^{31}$P-MRS reliability by way of a two-scan repeatability study in a cohort of healthy volunteers, 2) the hypothesis that bioenergetic abnormalities are present prior to cognitive impairment in early stage AD by measuring $^{31}$P-MRS in healthy, cognitively normal middle-aged adults at risk for AD (based on family history or genotype), 3) the relationship between bioenergetics and amyloid deposition measured with PET, and 4) the relationship between age and regional brain energy metabolism (Forester et al., 2010; Longo et al., 1993; Schmitz et al., 2018; Rietzler et al., 2021).

2. Methods

2.1. Subjects

The study was fully Health Insurance Portability and Accountability Act–compliant and the New York University Grossman School of Medicine Institutional Review Board approved the protocol. Community-residing subjects were scanned after providing informed consent and were compensated for their participation. The methods were carried out in accordance with Food and Drug Administration guidelines.

$^{31}$P-MRS repeatability cohort.—We assessed $^{31}$P-MRS repeatability by scanning 13 participants (6 females, min/max age: 23/59 years, age = 40.1 ± 13.5 years); 10 of whom were scanned on two separate days to measure inter-day repeatability (average duration between the two scans was 14.6 ± 18.4 days, ranging from 1 to 60 days) and 3 that were scanned two times on the same day to measure intra-day repeatability (interscan interval approximately 5 min).

$^{31}$P-MRS AD high-risk cohort.—Twenty individuals at high risk of AD due to a first-degree family history of late-onset (after 60 years of age) AD and/or positive apolipoprotein E4 (ApoE4) genotype were enrolled (17 females, min/max age: 38/67 years, age = 54.2 ± 7.5 years) (Table 1). These individuals had previously participated in a clinical study at our Center during which FDG-PET and $^{11}$C-Pittsburgh Compound B (PiB) PET evaluations (Murray et al., 2014) were carried out. The duration between the PET and $^{31}$P scans was 3.8 ± 1.4 years (minimum = 2.0, maximum = 6.4 years). Individuals with current or past...
conditions that may affect brain structure and metabolism such as stroke/cerebrovascular
disease, diabetes, head trauma, neurodegenerative disease, depression, hydrocephalus,
and intracranial masses on MRI, or use of psychoactive medications or steroids were
excluded. All subjects had education ≥ 12 years, Clinical Deterioration Rating = 0, Global
Deterioration Scale ≤ 2, Modified Hachinski Ischemia Scale < 4 and Mini-Mental State
Examination ≥ 26. All subjects had normal cognitive test performance relative to appropriate
reference values for age and education (Mosconi et al., 2007, 2010). These cognitive tests
were performed both at the time of the PET scans as well as at the time of the \(^{31}\)P-MRS
scans. Only individuals, who based on standardized family history questionnaires, had a
positive family history of late AD were included (Mosconi et al., 2009, 2010).

\(^{31}\)P-MRS versus aging cohort.—To study the relationship of age with the metabolites
measured using \(^{31}\)P-MRS the AD high-risk cohort and repeatability cohort were combined
into a single cross-sectional cohort (N = 33, 23 females, age = 48.6 ± 12.3 years; range
23–67 years).

2.2. MRI and \(^{31}\)P-MRS protocol for all participants

The MRI experiments were performed on a 3 T system (MAGNETOM Prisma, Siemens
Healthcare, Erlangen, Germany) with an investigational multi-nuclear \((^{31}\)P/\(^{1}\)H), transmit/
receive radiofrequency coil array. The device consists of two interleaved eight-channel
arrays for each nucleus (8 tuned to 49.9 MHz for \(^{31}\)P and 8–123.2 MHz for \(^{\text{H}}\)). Both arrays
encompass the head and are approximately 25 cm long to provide whole-brain coverage.

The \(^{31}\)P spectra were measured using a product 3D CSI sequence with elliptically weighted
k-space sampling and the following parameters: TE = 2.3 ms, TR = 2000 ms, flip angle
= 55°, acquired voxel size = 30 mm isotropic (zero filled for reconstruction at 15 mm
isotropic resolution), bandwidth = 2000 Hz, number of signal averages = 25, and acquisition
time = 23 min. Voxel-wise metabolite ratios PCr/\(\alpha\)-ATP, Pi/\(\alpha\)-ATP, and PME/PDE were
quantified using AMARES (Vanhamme et al., 1997) within the JMRUI software package
and co-registered to enable anatomic analysis using FreeSurfer software. (For simplicity,\(\alpha\)-ATP is referred to as ATP in this study.) We estimated voxel-wise pH values from the
chemical shift between Pi and PCr as described by the modified Henderson-Hasselbalch
equation (Taylor et al., 1983). The metabolite and pH maps were interpolated to match
the 1 mm isotropic resolution of co-registered \(^{1}\)H magnetization prepared rapid gradient
echo (MPRAGE) images that were acquired in the same examination with the following
parameters: TE = 2.7 ms, TI = 900 ms, TR = 2100 ms, flip angle = 8°, voxel size = 1
mm isotropic, bandwidth = 260 Hz/pixel, parallel imaging undersampling factor = 2, and
acquisition time = 4:02 min. The MPRAGE images were automatically segmented using
FreeSurfer software (Reuter et al., 2012) to establish the individual brain volumes-of-interest
in which \(^{31}\)P data are reported. We additionally report measurements in the Alzheimer’s
vulnerable meta region (Landau et al., 2011) that included the left angular gyrus, right
angular gyrus, bilateral posterior cingulate, and bilateral inferior temporal gyrus.
2.3. PET in the AD high-risk cohort

PET scans were acquired in 3D-mode on an LS Discovery [G.E. Medical Systems, Milwaukee, WI; 5.4 mm FWHM, 30 cm FOV] or a BioGraph PET/CT scanner [Siemens, Knoxville, TN; 1 mm FWHM, 25 cm FOV] following standardized procedures (Murray et al., 2014; Mosconi et al., 2007, 2010, 2013). Briefly, before PET imaging, an antecubital venous line was placed for isotope injection. Subjects rested with eyes open and ears unplugged in the quiet and dimly lit scan room. Subjects were positioned in the scanner using laser light beams for head alignment approximately 60 min after injection of 15 mCi of PiB. Total PiB scan time was 90 min (Mosconi et al., 2010, 2013). The FDG scan procedure started 30 min after the PiB scan or on a separate day. After an overnight fast, subjects were injected with 5 mCi of FDG, positioned in the scanner 35 min after injection, and scanned for 20 min. Prior to PET, a CT scan was acquired for attenuation correction. All images were corrected for photon attenuation, scatter, and radioactive decay, and reconstructed into a 512 × 512 matrix. The higher resolution (1 mm) scans were degraded to match the resolution of the LS Discovery scans using uniform resolution smoothing parameters (Joshi et al., 2009).

2.4. Statistical analysis

Statistical analyses were performed in MATLAB software (version 2020b, MathWorks, Natick, MA). We report $^{31}$P metabolite ratios PCr/ATP, Pi/ATP, PME/PDE and pH. The coefficient of variation for the repeatability study is reported as: $CV = 1 / N \sum_{i=1}^{N} \sigma_i / \mu_i$, where $\sigma_i, \mu_i$ are the standard deviation and mean of a given $^{31}$P measurement over 2 scans and $i$ is the subject index. Linear regression modeling was used to determine the relationship between: $^{31}$P measurements and whole brain tracer uptake and between whole brain tracer uptake and age in the AD high-risk cohort. Linear regression was also used to determine the relationship between $^{31}$P measurements and age in the cross-sectional cohort. Regional differences in $^{31}$P measurements in the AD high-risk cohort were determined using Dunn’s multiple comparison tests. Statistical significance was set at 1% (P < 0.01). Tests in which 0.01 ≤ P < 0.05 were considered to indicate a trend. All tests are reported without regard to sex or brain region size due to the exploratory nature of the study. For the 13 participants that were scanned twice, the mean $^{31}$P measurement values were incorporated into the age-dependent regressions.

2.5. Data availability

The MRI data generated for the study are available from the corresponding author with a formal sharing agreement to protect patient privacy.

3. Results

Fig. 1 shows a representative $^{31}$P spectrum in which excellent metabolite delineation can be observed. Table 2 lists metabolite ratio and pH measurement repeatability results. For 10 subjects scanned on different days, the average coefficient of variation in the AD meta region was 5.0% for PCr/ATP, 7.3% for Pi/ATP, 4.5% for PME/PDE, and 0.09% for pH.
For 3 subjects scanned on the same day, the average coefficient of variation in the AD meta region was 1.4% for PCr/ATP, 5.1% for Pi/ATP, 1.6% for PME/PDE, and 0.11% for pH.

In the AD high-risk cohort, metabolite ratios in the AD meta region PCr/ATP, and Pi/ATP showed significant inverse associations with FDG uptake (P < 0.01), while PME/PDE showed a trend toward association (P = 0.018) (see Table 3 and Fig. 2). FDG uptake was also inversely associated with: PCr/ATP in the inferior parietal lobe, inferior temporal cortex, and thalamus; Pi/ATP in the inferior parietal lobe, inferior temporal cortex, and superior temporal cortex; PME/PDE showed a trend toward association in the inferior temporal cortex, and superior temporal cortex (0.01 ≤ P < 0.05). No association in any brain region was observed between pH and whole brain FDG uptake (P > 0.1) or between 31P metabolic ratios and whole brain PiB uptake (P > 0.1, Supplementary Table 1). (A trend in precuneus pH and PiB uptake was observed P = 0.045, while pH versus PiB in other regions were uncorrelated.) All individuals were PiB negative defined by a tracer uptake value below 1.42 (Vlassenko et al., 2016).

Table 4 lists the linear regression analysis results for regional correlation between metabolite ratios and pH and age for the cross-sectional cohort (N = 33). The ratio PCr/ATP showed positive age-dependency in all regions in the analysis (P < 0.01) except the inferior parietal lobe and inferior temporal cortex, in which trends were observed (0.01 ≤ P < 0.05). The slope regression coefficient was 0.0063 units per year in the AD meta region.

To account for the interval between the PET and 31P scans, we defined time-corrected PCr/ATP as: PCr/ATP* = PCr/ATP − α × d, where α is the regionally-dependent slope in Table 4 and d the subject-dependent duration between scans in Table 1. Other 31P measurements were not corrected because of their stability as a function of age (Table 4). Linear regression analysis between PCr/ATP* and tracer uptake (Table 5) is similar to that between uncorrected PCr/ATP and tracer uptake in Table 3 and Supplementary Table 1.

Table 6 lists average metabolite ratios and pH values for the AD high-risk cohort in each brain region. The metabolite ratios PCr/ATP and PME/PDE along with pH showed regional differences, whereas Pi/ATP was stable across all regions (Tables 7-10). Supplementary Table 2 and Supplementary Table 3 list average metabolite ratios and pH values in the cross-sectional and repeatability cohorts.

### 4. Discussion

A strength of this study was its demonstration of excellent repeatability in 31P-MRS measurements; most regional metabolite ratio and pH inter- and intra-day CVs were well below 10% (Table 2). For comparison, Lagemaat et al. found 8.0% CV for PCr/ATP in a test-retest study without participant repositioning using an approximately 8 min acquisition protocol at 7 T with 12 mL nominal voxels that were enlarged to 38 mL due to filtering and undersampling (Lagemaat et al., 2016). While we did not record lifestyle information that could potentially cause day-to-day metabolic variability, the low inter-day CVs in the current study suggest that such factors are unlikely to confound measurements conducted within a relatively short timespan.
As pointed out by others, $^{31}$P-MRS measurements are affected by spatially variable transmit and receive field amplitudes, making it difficult to quantify metabolites in absolute terms (Rietzler et al., 2021; Meyerspeer et al., 2020). To alleviate this issue, metabolite ratios are often reported because they provide built-in normalization. Similarly, pH is determined by spectral relationships among metabolites, which eliminates sensitivity to field amplitude. Nonetheless, a range of PCR/ATP values are found in the literature. As a starting point for discussion, using the regression coefficients in Table 3 we calculated PCR/ATP of 1.2 in the AD meta region for a 49 year-old individual (selected to match the average age in the Rietzler et al. study), compared to 1.2 to 1.5 depending on region and sex in Rietzler et al. (2021), 1.7 in Schmitz et al. (2018), 1.4 to 1.6 in papers by Hattingen et al., 2009a, 2011, and 0.8 in Longo et al. (1993). While PCR/ATP is certainly influenced by study variables such as voxel size and position and cohort characteristics, a more likely explanation for the relatively low PCR/ATP value reported in this study is incomplete magnetization recovery due to the 2 s repetition time that was selected to accommodate a reasonable acquisition time (note that PCR longitudinal relaxation time is approximately 2.5 s at 3 T$^{27}$).

In the AD high-risk cohort we observed an inverse relationship between FDG-SUV uptake levels and metabolite ratios PCR/ATP and Pi/ATP in several brain regions, including the AD meta region (Table 3). Elevated PCR/ATP levels in mild-AD patients compared to age matched controls have been recently reported in Rijpma et al. (2018). Our results appear to be consistent with those findings, which suggest that decreased levels of glucose uptake are accompanied by redistribution in the content of metabolites involved in the creatine kinase equilibrium (Du et al., 2007). On the other hand, Das et al. (2021) observed lower PCR/ATP and Pi/ATP in the temporal lobe of individuals with amnestic mild cognitive impairment compared to controls and postulated that such trends may be indicative of a transitory cellular energy crisis that drives disease progression.

While there was a trend toward an inverse relationship between PME/PDE and FDG-SUV, this did not reach statistical significance, and further support the results in Rijpma et al. (2018) who did not observe group differences in mild-AD and age-matched controls in terms of phospholipid metabolite levels.

The association between FDG-SUV and $^{31}$P-MRS measured metabolites observed in this study appears to be consistent with Hu et al. who showed increased Pi/ATP in the temporoparietal cortex and reduced FDG in posterior parietal and temporal cortical grey matter in Parkinson’s disease patients (Hu et al., 2000). In the current study, the limitation of the timing between FDG-SUV and $^{31}$P-MRS scans (up to 6.4 years difference) requires us to interpret the results with caution. Nonetheless, Table 5 shows strong correlations between time-corrected PCR/ATP and FDG-SUV, while Table 4 shows that other $^{31}$P metabolites can be expected to remain relatively stable over time. Taken together, these data imply that $^{31}$P-MRS could provide insight on early changes in brain energetics in individuals at high risk for developing AD.

The $^{31}$P-MRS measurements showed a number of regional differences (Tables 7-10). One interesting observation was elevated PCR/ATP, depressed PME/PDE, and elevated pH in the temporal cortices that could respectively indicate reduced ATP utilization, cellular
membrane turnover, and glycolytic metabolism. The temporal cortices have been implicated in AD for loss of receptor function (Martin-Ruiz et al., 1999; Stokes and Hawthorne, 1987) and increased oxidative stress (Palmer and Burns, 1994), which add credence to the associations observed in this study. However, it is worth noting that elevated PCr/ATP, depressed PME/PDE, and elevated pH trends in the temporal cortices were present in both the AD high-risk cohort (Table 6) and the repeatability cohort (Supplementary Table 2), potentially suggesting a pattern of topographic predisposition to AD rather than a robust association of their presence with AD.

Abnormal metabolite levels have been observed in other neurodegenerative diseases such as Parkinson’s disease and multiple system atrophy (Martin, 2007). In accord with (Hu et al., 2000), others have shown that individuals with Parkinson’s disease have decreased high-energy phosphate levels in the visual cortex following visual activation (Rango et al., 2006), increased Pi in the occipital and frontal lobes (Barbiroli et al., 1999; Montagna et al., 1993), decreased ATP in the putamen and in the midbrain (Hattingen et al., 2009b), and decreased PCr in the putamen (Hattingen et al., 2011). However, the literature is conflicting. Hoang et al. reported normal metabolite levels in the putamen and parietal and occipital lobes (Hoang et al., 1998), while Weiduschat et al. observed no metabolic differences between early stage Parkinson’s and age-matched controls (Weiduschat et al., 2015). Indeed, a review article published in 2019 by Dossi et al. concluded that data from 10 $^{31}$P-MRS Parkinson’s studies are sparse and sometimes contrasting (Dossi et al., 2019).

We observed age-dependent PCr/ATP increases throughout the brain (Table 4), which is in agreement with the literature and suggests that ATP utilization decreases with age (Forester et al., 2010; Longo et al., 1993; Schmitz et al., 2018; Rietzler et al., 2021). We did not observe Pi/ATP age-dependency, which agrees with Longo et al. (1993) but contrasts with Rietzler et al. wherein Pi/ATP increased with age in a sex specific sub-cohort of 64 women (Rietzler et al., 2021). This disagreement may arise from a difference in cohort characteristics, as our study was not intended to explicitly evaluate the influence of sex on brain metabolism. While Rietzler and colleagues showed $^{31}$P metabolite ratio differences in several brain regions with respect to sex, the role of sex specific risk factors in AD is currently unclear (Mielke, 2018; Nebel et al., 2018). Jack et al. showed no sex differences in amyloid beta, tau burden, or neurodegeneration in cognitively normal individuals (Jack et al., 2017). However other studies showed that women with mild cognitive impairment had higher atrophy rates and faster cognitive decline than men (Holland et al., 2014; Hua et al., 2010; Lin et al., 2015).

We found no pH age-dependency. The literature on this point is conflicting; Forester et al. reported a negative correlation (Forester et al., 2010) and Longo et al. reported a positive correlation (Longo et al., 1993). While we did not observe a relationship between tracer uptake and age, it is important to point out that the AD high-risk cohort had a relatively narrow age range (min/max age: 38/67 years, age = 54.2 ± 7.5 years), making it difficult to evaluate age as an explanatory variable. Others have shown that FDG uptake in the anterior cingulate cortex, posterior cingulate cortex/precuneus, and lateral parietal cortex decreases with age (Ishibashi et al., 2018), which is consistent with age-dependent PCr/ATP increases reported in the current study.
The $^{31}$P data in this study was acquired with 3-cm isotropic voxels, which were linearly interpolated to 1-mm in order to perform anatomic analysis. This can give rise to partial volume effects that may not be random and can result in systematic bias in specific brain regions. One method to help address partial volume effects involves the use of high-resolution anatomical prior information from concurrent $^1$H-MRI to guide $^{31}$P image reconstruction (Rink et al., 2017). However, its impact on $^{31}$P brain imaging has not yet been determined.

A natural extension of this work will be to explore simultaneous MRI and PET imaging. Whole-body PET/MRI systems have become available during the past decade but research has focused almost exclusively on proton MRI applications, whereas multi-nuclear MRS (Hansen et al., 2016) such as $^{13}$C and $^{31}$P provides access to metabolic markers associated with early stage AD. One advantage of simultaneous $^{31}$P-MRS and PET is that physiological variation that may occur in separate examinations would be eliminated, mitigating spurious findings related to physiologic fluctuations between separate measurements (i.e., cerebral activation, cogitation, diurnal or circadian effects, post-prandial effects, etc.). One might speculate that correlation between FDG-SUV and $^{31}$P-MRS would be even stronger during concurrent scans than was observed in this study. In addition, dual-tuned PET-compatible radiofrequency coils would enable the simultaneous study of brain energetics together with amyloid/tau PET, potentially providing additional predictive information than that available from an individual tracer.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Representative $^{31}$P spectra acquired in a single 3.4 mL voxel within a whole-brain 3D CSI acquisition (23 min) in a 23-year-old female subject. The boxes in the three-plane proton MPRAGE images (left inset) delineate the voxel location.
Fig. 2.
Metabolite ratios in the Alzheimer’s vulnerable meta region were inversely associated with FDG uptake in the AD high-risk cohort (N = 20). P < 0.01 for PCr/ATP versus FDG and Pi/ATP versus FDG and 0.01 ≤ P < 0.05 for PME/PDE versus FDG. The linear regression results are listed in Table 3.
Table 1

Subject characteristics in the AD high-risk cohort.

| Subject | Age at $^{31}$P scan (years) | ApoE4 status | AD family history | Duration between PET and $^{31}$P scans (years) |
|---------|-------------------------------|--------------|-------------------|-----------------------------------------------|
| 1       | 49                            | 0            | Maternal and paternal | 4.1                                           |
| 2       | 53                            | N/A          | Maternal          | 3.1                                           |
| 3       | 49                            | +            | Paternal          | 5.7                                           |
| 4       | 49                            | +            | Maternal (grandmother and aunt) | 3.9                                           |
| 5       | 64                            | 0            | Paternal          | 2.8                                           |
| 6       | 50                            | 0            | Maternal grandmother (great aunt) | 3.0                                           |
| 7       | 62                            | +            | Maternal          | 3.3                                           |
| 8       | 60                            | +            | Maternal          | 2.4                                           |
| 9       | 52                            | +            | Paternal and paternal grandparents | 2.0                                           |
| 10      | 53                            | 0            | Paternal          | 2.4                                           |
| 11      | 48                            | 0            | Maternal and paternal | 2.7                                           |
| 12      | 55                            | +            | Paternal and paternal grandmother | 3.4                                           |
| 13      | 46                            | +            | Maternal (2 of 11 siblings have AD) | 6.4                                           |
| 14      | 55                            | 0            | Maternal and paternal | 5.5                                           |
| 15      | 67                            | 0            | Maternal          | 5.5                                           |
| 16      | 63                            | 0            | Maternal aunt     | 3.2                                           |
| 17      | 59                            | N/A          | Maternal, maternal grandmother, great maternal aunt | 3.4                                           |
| 18      | 38                            | +            | Paternal          | 2.8                                           |
| 19      | 64                            | 0            | Paternal, paternal uncle, 3 paternal cousins | 5.2                                           |
| 20      | 48                            | 0            | Maternal grandmother | 6.1                                           |

ApoE4: apolipoprotein E4.

N/A: not available.
Table 2

Coefficients of variation of regional $^{31}$P measurements in 10 participants scanned on 2 separate days (inter-day) and in 3 participants scanned 2 times on the same day (intra-day).

| Region                     | PCr/ATP | Pi/ATP | PME/PDE | pH  |
|----------------------------|---------|--------|---------|-----|
|                            | Inter   | Intra  | Inter   | Intra| Inter | Intra | Inter | Intra |
| AD meta region             | 5.0     | 1.4    | 7.3     | 5.1  | 4.5   | 1.6   | 0.09  | 0.11 |
| Inferior parietal lobe     | 4.2     | 1.7    | 8.8     | 7.5  | 4.5   | 1.2   | 0.09  | 0.12 |
| Inferior temporal cortex   | 6.3     | 3.6    | 10.7    | 3.5  | 10.7  | 10.1  | 0.10  | 0.09 |
| Middle frontal gyrus       | 6.0     | 2.0    | 7.7     | 4.9  | 6.1   | 3.9   | 0.09  | 0.04 |
| Posterior cingulate cortex | 5.2     | 4.1    | 7.3     | 6.9  | 4.3   | 5.2   | 0.10  | 0.06 |
| Precuneus                  | 4.3     | 4.0    | 8.0     | 3.9  | 5.2   | 4.5   | 0.12  | 0.09 |
| Prefrontal cortex          | 8.3     | 2.4    | 6.6     | 1.7  | 6.4   | 6.4   | 0.06  | 0.05 |
| Superior temporal cortex   | 7.2     | 7.3    | 6.4     | 15.2 | 6.9   | 6.4   | 0.08  | 0.17 |
| Thalamus                   | 5.7     | 6.0    | 8.4     | 6.6  | 6.0   | 5.0   | 0.11  | 0.08 |
### Table 3

Linear regression results for regional $^{31}$P measurements with whole brain FDG SUV in the AD high-risk cohort (N = 20). Significant correlations ($P < 0.01$) are shown in bold. Trends ($0.01 \leq P < 0.05$) are italicized.

| Regression variables | $^{31}$P-MRS Region          | $\alpha$ | $\beta$ | $R^2$ | $P$    |
|----------------------|-------------------------------|----------|---------|-------|--------|
| PCr/ATP vs. FDG      | AD meta region                | -1.84    | 3.22    | 0.361 | 0.0050 |
| PCr/ATP vs. FDG      | Inferior parietal lobe        | -1.49    | 2.73    | 0.329 | 0.0082 |
| PCr/ATP vs. FDG      | Inferior temporal cortex      | -3.04    | 4.69    | 0.344 | 0.0066 |
| PCr/ATP vs. FDG      | Middle frontal gyrus          | -0.66    | 1.81    | 0.070 | 0.2599 |
| PCr/ATP vs. FDG      | Posterior cingulate cortex    | -0.56    | 1.66    | 0.078 | 0.2328 |
| PCr/ATP vs. FDG      | Precuneus                     | -1.03    | 2.21    | 0.219 | 0.0375 |
| PCr/ATP vs. FDG      | Inferior parietal lobe        | -0.90    | 2.07    | 0.065 | 0.2774 |
| PCr/ATP vs. FDG      | Superior temporal cortex      | -2.26    | 3.77    | 0.307 | 0.0113 |
| PCr/ATP vs. FDG      | Thalamus                      | -1.76    | 3.02    | 0.383 | 0.0036 |
| Pi/ATP vs. FDG       | AD meta region                | -0.98    | 1.40    | 0.409 | 0.0024 |
| Pi/ATP vs. FDG       | Inferior parietal lobe        | -0.93    | 1.38    | 0.325 | 0.0087 |
| Pi/ATP vs. FDG       | Inferior temporal cortex      | -1.44    | 1.86    | 0.580 | 0.0001 |
| Pi/ATP vs. FDG       | Middle frontal gyrus          | -0.46    | 0.82    | 0.171 | 0.0698 |
| Pi/ATP vs. FDG       | Posterior cingulate cortex    | -0.32    | 0.70    | 0.055 | 0.3175 |
| Pi/ATP vs. FDG       | Precuneus                     | -0.60    | 1.04    | 0.113 | 0.1473 |
| Pi/ATP vs. FDG       | Prefrontal cortex             | -0.49    | 0.86    | 0.168 | 0.0725 |
| Pi/ATP vs. FDG       | Superior temporal cortex      | -1.09    | 1.50    | 0.479 | 0.0007 |
| Pi/ATP vs. FDG       | Thalamus                      | -0.75    | 1.16    | 0.229 | 0.0328 |
| PME/PDE vs. FDG      | AD meta region                | -1.49    | 2.89    | 0.273 | 0.0182 |
| PME/PDE vs. FDG      | Inferior parietal lobe        | -1.05    | 2.56    | 0.121 | 0.1335 |
| PME/PDE vs. FDG      | Inferior temporal cortex      | -2.23    | 3.35    | 0.260 | 0.0217 |
| PME/PDE vs. FDG      | Middle frontal gyrus          | -0.36    | 1.84    | 0.012 | 0.6456 |
| PME/PDE vs. FDG      | Posterior cingulate cortex    | -0.31    | 1.96    | 0.015 | 0.6037 |
| PME/PDE vs. FDG      | Precuneus                     | -1.04    | 2.63    | 0.146 | 0.0961 |
| PME/PDE vs. FDG      | Prefrontal cortex             | -0.43    | 1.86    | 0.028 | 0.4769 |
| PME/PDE vs. FDG      | Superior temporal cortex      | -1.82    | 3.13    | 0.272 | 0.0185 |
| PME/PDE vs. FDG      | Thalamus                      | -1.30    | 2.79    | 0.195 | 0.0511 |
| pH vs. FDG           | AD meta region                | -0.08    | 7.09    | 0.127 | 0.1238 |
| pH vs. FDG           | Inferior parietal lobe        | -0.05    | 7.05    | 0.021 | 0.5412 |
| pH vs. FDG           | Inferior temporal cortex      | -0.14    | 7.18    | 0.128 | 0.1210 |
| pH vs. FDG           | Middle frontal gyrus          | -0.09    | 7.09    | 0.121 | 0.1326 |
| pH vs. FDG           | Posterior cingulate cortex    | -0.11    | 7.10    | 0.137 | 0.1085 |
| pH vs. FDG           | Precuneus                     | -0.04    | 7.03    | 0.008 | 0.7066 |
| pH vs. FDG           | Prefrontal cortex             | -0.10    | 7.11    | 0.091 | 0.1961 |
| pH vs. FDG           | Superior temporal cortex      | -0.09    | 7.11    | 0.091 | 0.1965 |
| pH vs. FDG           | Thalamus                      | -0.11    | 7.10    | 0.118 | 0.1376 |

$\alpha$: linear regression slope; $\beta$: linear regression intercept. $R^2$: linear regression coefficient of determination.
Table 4
Linear regression results for regional $^{31}$P measurements with age in the cross-sectional cohort (N = 33). Significant correlations (P < 0.01) are shown in bold. Trends (0.01 ≤ P < 0.05) are italicized.

| Regression variables | $^{31}$P-MRS Region       | $\alpha$ (years$^{-1} \times 10^{-4}$) | $\beta$ | $R^2$ | P      |
|----------------------|-----------------------------|----------------------------------------|---------|------|--------|
| PCr/ATP vs. age      | AD meta region              | 62.88                                  | 0.90    | 0.259| **0.0025** |
| PCr/ATP vs. age      | Inferior parietal           | 45.12                                  | 0.90    | 0.181| **0.0134** |
| PCr/ATP vs. age      | Inferior temporal cortex    | 94.98                                  | 0.93    | 0.188| **0.0117** |
| PCr/ATP vs. age      | Middle frontal gyms         | 52.11                                  | 0.82    | 0.258| **0.0026** |
| PCr/ATP vs. age      | Posterior cingulate cortex  | 36.29                                  | 0.85    | 0.223| **0.0055** |
| PCr/ATP vs. age      | Precuneus                   | 40.60                                  | 0.88    | 0.227| **0.0050** |
| PCr/ATP vs. age      | Prefrontal cortex           | 55.07                                  | 0.80    | 0.206| **0.0079** |
| PCr/ATP vs. age      | Superior temporal cortex    | 75.37                                  | 0.95    | 0.222| **0.0057** |
| PCr/ATP vs. age      | Thalamus                    | 51.48                                  | 0.86    | 0.219| **0.0061** |
| Pi/ATP vs. age       | AD meta region              | 6.35                                   | 0.32    | 0.020| 0.4293 |
| Pi/ATP vs. age       | Inferior parietal           | 7.52                                   | 0.33    | 0.023| 0.3959 |
| Pi/ATP vs. age       | Inferior parietal           | 8.78                                   | 0.28    | 0.025| 0.3819 |
| Pi/ATP vs. age       | Middle frontal gyms         | 0.23                                   | 0.33    | 0.000| 0.9716 |
| Pi/ATP vs. age       | Posterior cingulate cortex  | 1.12                                   | 0.35    | 0.001| 0.8769 |
| Pi/ATP vs. age       | Precuneus                   | 8.60                                   | 0.33    | 0.025| 0.3818 |
| Pi/ATP vs. age       | Prefrontal cortex           | -0.36                                  | 0.34    | 0.000| 0.9568 |
| Pi/ATP vs. age       | Superior temporal cortex    | 4.63                                   | 0.30    | 0.010| 0.5734 |
| Pi/ATP vs. age       | Thalamus                    | 4.15                                   | 0.33    | 0.008| 0.6170 |
| PME/PDE vs. age      | AD meta region              | 5.80                                   | 1.27    | 0.004| 0.7348 |
| PME/PDE vs. age      | Inferior parietal           | 27.79                                  | 1.27    | 0.068| 0.1420 |
| PME/PDE vs. age      | Inferior temporal cortex    | -16.39                                 | 1.08    | 0.009| 0.5941 |
| PME/PDE vs. age      | Middle frontal gyms         | 10.46                                  | 1.38    | 0.008| 0.6187 |
| PME/PDE vs. age      | Posterior cingulate cortex  | 4.08                                   | 1.60    | 0.002| 0.8074 |
| PME/PDE vs. age      | Precuneus                   | 22.42                                  | 1.40    | 0.054| 0.1948 |
| PME/PDE vs. age      | Prefrontal cortex           | 3.39                                   | 1.39    | 0.002| 0.8194 |
| PME/PDE vs. age      | Superior temporal cortex    | 9.15                                   | 1.12    | 0.006| 0.6633 |
| PME/PDE vs. age      | Thalamus                    | -6.13                                  | 1.44    | 0.004| 0.7408 |
| pH vs. age           | AD meta region              | 1.08                                   | 7.00    | 0.021| 0.4162 |
| pH vs. age           | Inferior parietal lobe      | 0.27                                   | 7.00    | 0.001| 0.8870 |
| pH vs. age           | Inferior temporal cortex    | 2.07                                   | 7.02    | 0.027| 0.3579 |
| pH vs. age           | Middle frontal gyms         | -0.34                                  | 7.00    | 0.001| 0.8384 |
| pH vs. age           | Posterior cingulate cortex  | -0.62                                  | 6.99    | 0.004| 0.7400 |
| pH vs. age           | Precuneus                   | -0.21                                  | 7.00    | 0.000| 0.9246 |
| pH vs. age           | Prefrontal cortex           | -0.30                                  | 7.00    | 0.001| 0.8832 |
| pH vs. age           | Superior temporal cortex    | 1.15                                   | 7.01    | 0.011| 0.5565 |
| pH vs. age           | Thalamus                    | -0.15                                  | 6.99    | 0.000| 0.9320 |

$\alpha$: linear regression slope. $\beta$: linear regression intercept. $R^2$: linear regression coefficient of determination.
Table 5

Linear regression results for regional, time corrected PCr/ATP ratios with whole brain FDG and PiB SUV in the AD high-risk cohort (N = 20). Significant correlations (P < 0.01) are shown in bold. Trends (0.01 ≤ P < 0.05) are italicized.

| Regression variables | MRS Region | α     | β     | R²   | P       |
|----------------------|------------|-------|-------|------|---------|
| PCr/ATP<sup>a</sup> vs. FDG | AD meta region | −1.91 | 3.26  | 0.377| 0.0040  |
|                      | Inferior parietal lobe | −1.54 | 2.77  | 0.346| 0.0063  |
|                      | Inferior temporal cortex | −3.14 | 4.76  | 0.357| 0.0054  |
|                      | Middle frontal gyms | −0.71 | 1.85  | 0.079| 0.2301  |
|                      | Posterior cingulate cortex | −0.60 | 1.69  | 0.088| 0.2033  |
|                      | Precuneus | −1.07 | 2.24  | 0.255| 0.0304  |
|                      | Prefrontal cortex | −0.96 | 2.11  | 0.074| 0.2475  |
|                      | Superior temporal cortex | −2.34 | 3.83  | 0.318| 0.0097  |
|                      | Thalamus | −1.81 | 3.06  | 0.394| 0.0030  |
| PCr/ATP<sup>a</sup> vs. PiB | AD meta region | −0.14 | 1.37  | 0.006| 0.7474  |
|                      | Inferior parietal lobe | 0.09  | 1.03  | 0.003| 0.8128  |
|                      | Inferior temporal cortex | −0.13 | 1.53  | 0.002| 0.8654  |
|                      | Middle frontal gyms | −0.10 | 1.19  | 0.004| 0.7881  |
|                      | Posterior cingulate cortex | −0.12 | 1.17  | 0.010| 0.6700  |
|                      | Precuneus | 0.00  | 1.10  | 0.000| 0.9914  |
|                      | Prefrontal cortex | −0.36 | 1.45  | 0.029| 0.4745  |
|                      | Superior temporal cortex | −0.44 | 1.77  | 0.031| 0.4594  |
|                      | Thalamus | −0.25 | 1.38  | 0.021| 0.5396  |

<sup>a</sup> time-corrected.

α: linear regression slope. β: linear regression intercept. R²: linear regression coefficient of determination.
Table 6

Mean and standard deviations of $^{31}$P-MRS measurements in the AD high-risk cohort (N = 20).

| Region                  | PCR/ATP   | Pi/ATP    | PME/PDE  | pH       |
|-------------------------|-----------|-----------|----------|----------|
| AD meta region          | 1.24 ± 0.13 | 0.35 ± 0.07 | 1.29 ± 0.12 | 7.01 ± 0.01 |
| Inferior parietal lobe  | 1.13 ± 0.11 | 0.38 ± 0.07 | 1.43 ± 0.13 | 7.00 ± 0.01 |
| Inferior temporal cortex| 1.43 ± 0.23 | 0.32 ± 0.08 | 0.96 ± 0.19 | 7.03 ± 0.02 |
| Middle frontal gyrus    | 1.10 ± 0.11 | 0.33 ± 0.05 | 1.45 ± 0.14 | 6.99 ± 0.01 |
| Posterior cingulate cortex | 1.06 ± 0.09 | 0.36 ± 0.06 | 1.63 ± 0.11 | 6.98 ± 0.01 |
| Precuneus               | 1.11 ± 0.10 | 0.39 ± 0.08 | 1.52 ± 0.12 | 7.00 ± 0.02 |
| Prefrontal cortex       | 1.10 ± 0.15 | 0.34 ± 0.05 | 1.40 ± 0.11 | 7.00 ± 0.02 |
| Superior temporal cortex| 1.35 ± 0.18 | 0.33 ± 0.07 | 1.18 ± 0.15 | 7.01 ± 0.01 |
| Thalamus                | 1.13 ± 0.12 | 0.36 ± 0.07 | 1.40 ± 0.13 | 6.99 ± 0.01 |
Table 7

Regional PCr/ATP comparison in the AD high-risk cohort (N = 20). Significant regional differences (P < 0.01) are shown in bold. Trends (0.01 ≤ P < 0.05) are italicized. The differences between group means and the 99% confidence intervals for differences between group means are listed.

| Region 1             | Region 2                  | Lower Confidence Level | Difference | Upper Confidence Level | P      |
|----------------------|---------------------------|------------------------|------------|------------------------|--------|
| AD meta region       | Inferior parietal lobe    | −0.060                 | 0.108      | 0.275                  | 0.4801 |
| AD meta region       | Inferior temporal cortex  | −0.359                 | −0.192     | −0.025                 | **0.0012** |
| AD meta region       | Middle frontal gyrus      | −0.029                 | 0.138      | 0.305                  | 0.0862 |
| AD meta region       | Posterior cingulate cortex| 0.015                  | 0.183      | 0.350                  | **0.0027** |
| AD meta region       | Prefrontal cortex         | −0.032                 | 0.135      | 0.302                  | 0.1074 |
| AD meta region       | Superior temporal cortex  | −0.270                 | −0.103     | 0.064                  | 0.5765 |
| AD meta region       | Thalamus                  | −0.059                 | 0.108      | 0.275                  | 0.4719 |
| Inferior parietal lobe| Inferior temporal cortex  | −0.467                 | −0.300     | −0.133                 | < 1 × 10⁻⁴ |
| Inferior parietal lobe| Middle frontal gyrus      | −0.136                 | 0.031      | 0.198                  | 1.0000 |
| Inferior parietal lobe| Posterior cingulate cortex| −0.092                 | 0.075      | 0.242                  | 0.9750 |
| Inferior parietal lobe| Precuneus                 | −0.140                 | 0.027      | 0.194                  | 1.0000 |
| Inferior parietal lobe| Prefrontal cortex         | −0.133                 | 0.034      | 0.201                  | 1.0000 |
| Inferior parietal lobe| Superior temporal cortex  | −0.377                 | −0.210     | −0.043                 | **0.0002** |
| Inferior parietal lobe| Thalamus                  | −0.167                 | 0.000      | 0.167                  | 1.0000 |
| Inferior temporal cortex| Middle frontal gyrus      | 0.163                  | 0.330      | 0.497                  | < 1 × 10⁻⁴ |
| Inferior temporal cortex| Posterior cingulate cortex| 0.208                  | 0.375      | 0.542                  | < 1 × 10⁻⁴ |
| Inferior temporal cortex| Precuneus                 | 0.160                  | 0.327      | 0.494                  | < 1 × 10⁻⁴ |
| Inferior temporal cortex| Prefrontal cortex         | 0.166                  | 0.333      | 0.500                  | < 1 × 10⁻⁴ |
| Inferior temporal cortex| Superior temporal cortex  | −0.078                 | 0.089      | 0.256                  | 0.8333 |
| Inferior temporal cortex| Thalamus                  | 0.133                  | 0.300      | 0.467                  | < 1 × 10⁻⁴ |
| Middle frontal gyrus| Posterior cingulate cortex| −0.123                 | 0.044      | 0.211                  | 1.0000 |
| Middle frontal gyrus| Precuneus                 | −0.170                 | −0.003     | 0.164                  | 1.0000 |
| Middle frontal gyrus| Prefrontal cortex         | −0.164                 | 0.003      | 0.170                  | 1.0000 |
| Middle frontal gyrus| Superior temporal cortex  | −0.408                 | −0.241     | −0.074                 | < 1 × 10⁻⁴ |
| Middle frontal gyrus| Thalamus                  | −0.197                 | −0.030     | 0.137                  | 1.0000 |
| Posterior cingulate cortex| Precuneus                 | −0.215                 | −0.048     | 0.119                  | 1.0000 |
| Posterior cingulate cortex| Prefrontal cortex         | −0.208                 | −0.041     | 0.126                  | 1.0000 |
| Posterior cingulate cortex| Superior temporal cortex  | −0.452                 | −0.285     | −0.118                 | < 1 × 10⁻⁴ |
| Posterior cingulate cortex| Thalamus                  | −0.242                 | −0.075     | 0.092                  | 0.9768 |
| Precuneus            | Prefrontal cortex         | −0.161                 | 0.006      | 0.173                  | 1.0000 |
| Precuneus            | Superior temporal cortex  | −0.405                 | −0.238     | −0.071                 | < 1 × 10⁻⁴ |
| Precuneus            | Thalamus                  | −0.194                 | −0.027     | 0.140                  | 1.0000 |
| Prefrontal cortex    | Superior temporal cortex  | −0.411                 | −0.244     | −0.077                 | < 1 × 10⁻⁴ |
| Prefrontal cortex    | Thalamus                  | −0.200                 | −0.033     | 0.134                  | 1.0000 |
| Region 1               | Region 2 | Lower Confidence Level | Difference | Upper Confidence Level | P       |
|-----------------------|----------|------------------------|------------|------------------------|---------|
| Superior temporal cortex | Thalamus | 0.044                  | 0.211      | 0.378                  | 0.0002  |
Table 8

Regional Pi/ATP comparison in the AD high-risk cohort (N = 20). No significant regional differences or trends were observed. The differences between group means and the 99% confidence intervals for differences between group means are listed.

| Region 1               | Region 2               | Lower Confidence level | Difference | Upper Confidence level | P       |
|------------------------|------------------------|------------------------|------------|------------------------|---------|
| AD meta region         | Inferior parietal      | −0.104                 | −0.025     | 0.054                  | 1.0000  |
| AD meta region         | Inferior temporal cortex | −0.046               | 0.033      | 0.111                  | 0.9922  |
| AD meta region         | Middle frontal gyrus   | −0.058                 | 0.021      | 0.099                  | 1.0000  |
| AD meta region         | Posterior cingulate cortex | −0.086               | −0.008     | 0.071                  | 1.0000  |
| AD meta region         | Precuneus              | −0.115                 | −0.036     | 0.043                  | 0.9701  |
| AD meta region         | Prefrontal cortex      | −0.062                 | 0.017      | 0.096                  | 1.0000  |
| AD meta region         | Superior temporal cortex | −0.052               | 0.026      | 0.105                  | 0.9998  |
| AD meta region         | Thalamus               | −0.084                 | −0.006     | 0.073                  | 1.0000  |
| Inferior parietal lobe | Inferior temporal cortex | −0.021               | 0.057      | 0.136                  | 0.2340  |
| Inferior parietal lobe | Middle frontal gyrus   | −0.033                 | 0.045      | 0.124                  | 0.7078  |
| Inferior parietal lobe | Posterior cingulate cortex | −0.061               | 0.017      | 0.096                  | 1.0000  |
| Inferior parietal lobe | Precuneus              | −0.090                 | −0.011     | 0.068                  | 1.0000  |
| Inferior parietal lobe | Prefrontal cortex      | −0.037                 | 0.042      | 0.120                  | 0.8460  |
| Inferior parietal lobe | Superior temporal cortex | −0.027               | 0.051      | 0.130                  | 0.4518  |
| Inferior parietal lobe | Thalamus               | −0.060                 | 0.019      | 0.098                  | 1.0000  |
| Inferior temporal cortex | Middle frontal gyrus   | −0.091                 | −0.012     | 0.067                  | 1.0000  |
| Inferior temporal cortex | Posterior cingulate cortex | −0.119               | −0.040     | 0.038                  | 0.8881  |
| Inferior temporal cortex | Precuneus              | −0.147                 | −0.068     | 0.010                  | 0.0527  |
| Inferior temporal cortex | Prefrontal cortex      | −0.094                 | −0.016     | 0.063                  | 1.0000  |
| Inferior temporal cortex | Superior temporal cortex | −0.085               | −0.006     | 0.073                  | 1.0000  |
| Inferior temporal cortex | Thalamus               | −0.117                 | −0.038     | 0.040                  | 0.9327  |
| Middle frontal gyrus   | Posterior cingulate cortex | −0.107               | −0.028     | 0.050                  | 0.9994  |
| Middle frontal gyrus   | Precuneus              | −0.135                 | −0.056     | 0.022                  | 0.2681  |
| Middle frontal gyrus   | Prefrontal cortex      | −0.082                 | −0.004     | 0.075                  | 1.0000  |
| Middle frontal gyrus   | Superior temporal cortex | −0.073               | 0.006      | 0.085                  | 1.0000  |
| Middle frontal gyrus   | Thalamus               | −0.105                 | −0.026     | 0.052                  | 0.9998  |
| Posterior cingulate cortex | Precuneus              | −0.107                 | −0.028     | 0.051                  | 0.9994  |
| Posterior cingulate cortex | Prefrontal cortex      | −0.054                 | 0.025      | 0.103                  | 1.0000  |
| Posterior cingulate cortex | Superior temporal cortex | −0.045               | 0.034      | 0.113                  | 0.9846  |
| Posterior cingulate cortex | Thalamus               | −0.077                 | 0.002      | 0.081                  | 1.0000  |
| Precuneus              | Prefrontal cortex      | −0.026                 | 0.053      | 0.131                  | 0.3978  |
| Precuneus              | Superior temporal cortex | −0.016               | 0.062      | 0.141                  | 0.1279  |
| Precuneus              | Thalamus               | −0.049                 | 0.030      | 0.109                  | 0.9980  |
| Prefrontal cortex      | Superior temporal cortex | −0.069               | 0.010      | 0.088                  | 1.0000  |
| Prefrontal cortex      | Thalamus               | −0.101                 | −0.023     | 0.056                  | 1.0000  |
| Superior temporal cortex | Thalamus               | −0.111                 | −0.032     | 0.047                  | 0.9936  |
Table 9

Regional PME/PDE comparison in the AD high-risk cohort (N = 20). Significant regional differences (P < 0.01) are shown in bold. Trends (0.01 ≤ P < 0.05) are italicized. The differences between group means and the 99% confidence intervals for differences between group means are listed.

| Region 1 | Region 2 | Lower Confidence Level | Difference | Upper Confidence Level | P       |
|----------|----------|------------------------|------------|------------------------|---------|
| AD meta region | Inferior parietal lobe | −0.297 | −0.136 | 0.025 | 0.0690 |
| AD meta region | Inferior temporal cortex | 0.173 | 0.334 | 0.494 | < 1 × 10⁻⁴ |
| AD meta region | Middle frontal gyrus | −0.318 | −0.158 | 0.003 | 0.0230 |
| AD meta region | Posterior cingulate cortex | −0.497 | −0.337 | −0.176 | < 1 × 10⁻⁴ |
| AD meta region | Precuneus | −0.385 | −0.225 | −0.064 | < 1 × 10⁻⁴ |
| AD meta region | Prefrontal cortex | −0.267 | −0.106 | 0.054 | 0.4228 |
| AD meta region | Superior temporal cortex | −0.051 | 0.110 | 0.270 | 0.3596 |
| AD meta region | Thalamus | −0.263 | −0.102 | 0.059 | 0.5074 |
| Inferior parietal lobe | Inferior temporal cortex | 0.309 | 0.470 | 0.630 | < 1 × 10⁻⁴ |
| Inferior parietal lobe | Middle frontal gyrus | −0.182 | −0.021 | 0.139 | 1.0000 |
| Inferior parietal lobe | Posterior cingulate cortex | −0.361 | −0.201 | −0.040 | 0.0003 |
| Inferior parietal lobe | Precuneus | −0.249 | −0.089 | 0.072 | 0.7894 |
| Inferior parietal lobe | Prefrontal cortex | −0.131 | 0.030 | 0.191 | 1.0000 |
| Inferior parietal lobe | Superior temporal cortex | 0.085 | 0.246 | 0.406 | < 1 × 10⁻⁴ |
| Inferior parietal lobe | Thalamus | −0.127 | 0.034 | 0.195 | 1.0000 |
| Inferior temporal cortex | Middle frontal gyrus | −0.652 | −0.491 | −0.330 | < 1 × 10⁻⁴ |
| Inferior temporal cortex | Posterior cingulate cortex | −0.831 | −0.670 | −0.509 | < 1 × 10⁻⁴ |
| Inferior temporal cortex | Precuneus | −0.719 | −0.558 | −0.397 | < 1 × 10⁻⁴ |
| Inferior temporal cortex | Prefrontal cortex | −0.601 | −0.440 | −0.279 | < 1 × 10⁻⁴ |
| Inferior temporal cortex | Superior temporal cortex | −0.385 | −0.224 | −0.063 | < 1 × 10⁻⁴ |
| Inferior temporal cortex | Thalamus | −0.596 | −0.436 | −0.275 | < 1 × 10⁻⁴ |
| Middle frontal gyrus | Posterior cingulate cortex | −0.340 | −0.179 | −0.018 | 0.0020 |
| Middle frontal gyrus | Precuneus | −0.228 | −0.067 | 0.094 | 0.9912 |
| Middle frontal gyrus | Prefrontal cortex | −0.109 | 0.051 | 0.212 | 0.9999 |
| Middle frontal gyrus | Superior temporal cortex | 0.106 | 0.267 | 0.428 | < 1 × 10⁻⁴ |
| Middle frontal gyrus | Thalamus | −0.105 | 0.055 | 0.216 | 0.9997 |
| Posterior cingulate cortex | Precuneus | −0.049 | 0.112 | 0.273 | 0.3176 |
| Posterior cingulate cortex | Prefrontal cortex | 0.070 | 0.230 | 0.391 | < 1 × 10⁻⁴ |
| Posterior cingulate cortex | Superior temporal cortex | 0.286 | 0.446 | 0.607 | < 1 × 10⁻⁴ |
| Posterior cingulate cortex | Thalamus | 0.074 | 0.235 | 0.395 | < 1 × 10⁻⁴ |
| Precuneus | Prefrontal cortex | −0.042 | 0.118 | 0.279 | 0.2215 |
| Precuneus | Superior temporal cortex | 0.174 | 0.334 | 0.495 | < 1 × 10⁻⁴ |
| Precuneus | Thalamus | −0.038 | 0.123 | 0.283 | 0.1724 |
| Prefrontal cortex | Superior temporal cortex | 0.055 | 0.216 | 0.377 | < 1 × 10⁻⁴ |
| Prefrontal cortex | Thalamus | −0.157 | 0.004 | 0.165 | 1.0000 |
| Region 1                  | Region 2   | Lower Confidence Level | Difference | Upper Confidence Level | P            |
|--------------------------|------------|------------------------|------------|------------------------|--------------|
| Superior temporal cortex | Thalamus   | −0.373                 | −0.212     | −0.051                 | < 1 × 10^{-4} |
Table 10
Regional pH comparison in the AD high-risk cohort (N = 20). Significant regional differences (P < 0.01) are shown in bold. Trends (0.01 ≤ P < 0.05) are italicized. The differences between group means and the 99% confidence intervals for differences between group means are listed.

| Region 1 | Region 2 | Lower Confidence level | Difference | Upper Confidence level | P       |
|----------|----------|------------------------|------------|------------------------|---------|
| AD meta region | Inferior parietal lobe | −0.010 | 0.006 | 0.023 | 0.9982 |
| AD meta region | Inferior temporal cortex | −0.035 | −0.018 | −0.002 | **0.0020** |
| AD meta region | Middle frontal gyrus | −0.003 | 0.014 | 0.030 | 0.0776 |
| AD meta region | Posterior cingulate cortex | 0.009 | 0.025 | 0.041 | < 1 × 10⁻⁴ |
| AD meta region | Precuneus | −0.005 | 0.011 | 0.027 | 0.3735 |
| AD meta region | Prefrontal cortex | −0.004 | 0.013 | 0.029 | 0.1376 |
| AD meta region | Superior temporal cortex | −0.022 | −0.006 | 0.011 | 0.9996 |
| AD meta region | Thalamus | 0.003 | 0.019 | 0.036 | **0.0007** |
| Inferior parietal lobe | Inferior temporal cortex | −0.041 | −0.024 | −0.008 | < 1 × 10⁻⁴ |
| Inferior parietal lobe | Middle frontal gyrus | −0.009 | 0.007 | 0.024 | 0.9688 |
| Inferior parietal lobe | Posterior cingulate cortex | 0.003 | 0.019 | 0.035 | **0.0011** |
| Inferior parietal lobe | Precuneus | −0.011 | 0.005 | 0.021 | 1.0000 |
| Inferior parietal lobe | Prefrontal cortex | −0.010 | 0.007 | 0.023 | 0.9946 |
| Inferior parietal lobe | Superior temporal cortex | −0.028 | −0.012 | 0.004 | 0.2330 |
| Inferior parietal lobe | Thalamus | −0.003 | 0.013 | 0.029 | 0.1150 |
| Inferior temporal cortex | Middle frontal gyrus | 0.016 | 0.032 | 0.048 | < 1 × 10⁻⁴ |
| Inferior temporal cortex | Posterior cingulate cortex | 0.027 | 0.043 | 0.060 | < 1 × 10⁻⁴ |
| Inferior temporal cortex | Precuneus | 0.013 | 0.029 | 0.046 | < 1 × 10⁻⁴ |
| Inferior temporal cortex | Prefrontal cortex | 0.015 | 0.031 | 0.047 | < 1 × 10⁻⁴ |
| Inferior temporal cortex | Superior temporal cortex | −0.004 | 0.012 | 0.029 | 0.1736 |
| Inferior temporal cortex | Thalamus | 0.021 | 0.038 | 0.054 | < 1 × 10⁻⁴ |
| Middle frontal gyrus | Posterior cingulate cortex | −0.005 | 0.011 | 0.028 | 0.3152 |
| Middle frontal gyrus | Precuneus | −0.019 | −0.003 | 0.014 | 1.0000 |
| Middle frontal gyrus | Prefrontal cortex | −0.017 | −0.001 | 0.016 | 1.0000 |
| Middle frontal gyrus | Superior temporal cortex | −0.036 | −0.019 | −0.003 | **0.0007** |
| Middle frontal gyrus | Thalamus | −0.011 | 0.006 | 0.022 | 0.9997 |
| Posterior cingulate cortex | Precuneus | −0.030 | −0.014 | 0.002 | 0.0612 |
| Posterior cingulate cortex | Prefrontal cortex | −0.029 | −0.012 | 0.004 | 0.1948 |
| Posterior cingulate cortex | Superior temporal cortex | −0.047 | −0.031 | −0.014 | < 1 × 10⁻⁴ |
| Posterior cingulate cortex | Thalamus | −0.022 | −0.006 | 0.011 | 0.9995 |
| Precuneus | Prefrontal cortex | −0.015 | 0.002 | 0.018 | 1.0000 |
| Precuneus | Superior temporal cortex | −0.033 | −0.017 | 0.000 | **0.0007** |
| Precuneus | Thalamus | −0.008 | 0.008 | 0.025 | 0.9063 |
| Prefrontal cortex | Superior temporal cortex | −0.035 | −0.019 | −0.002 | **0.0015** |
| Prefrontal cortex | Thalamus | −0.010 | 0.006 | 0.023 | 0.9962 |
| Region 1                  | Region 2    | Lower Confidence level | Difference | Upper Confidence level | P       |
|-------------------------|-------------|------------------------|------------|------------------------|---------|
| Superior temporal cortex| Thalamus    | 0.009                  | 0.025      | 0.041                  | $< 1 \times 10^{-4}$ |

*Note: The table compares the lower confidence level, difference, and upper confidence level for brain regions.*