Developing MR Biomarkers of Neurodegeneration: Applications in Preclinical Alzheimer's Disease

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Background

Alzheimer's disease (AD) is a leading cause of death and disability, with an estimated prevalence of 5.8 million in 2020 that is predicted to triple to nearly 14 million by 2060. The current medications can slow disease progression but cannot reverse it. Thus, there is a critical need for a biomarker profile of early-stage AD to not only improve disease screening and management, but also to aid the development of disease-modifying therapies.

Several cerebrospinal fluid (CSF) and imaging biomarkers have been established as surrogates of Aβ deposition ("A"), tau pathology ("T"), and neurodegeneration ["(N)"]. Unfortunately, much is still unknown about the pathophysiological mechanisms which underlie AT(N). This gap in knowledge can be addressed by using whole-brain proton magnetic resonance spectroscopy (^{1}H-MRS), a non-invasive in vivo imaging technique, to examine brain biochemistry in cognitively normal elderly. ^{1}H-MRS does not require injections of radiotracers and can simultaneously yield localized markers for membrane turnover, cellular energy, cell signaling, astrocytosis, and neuronal integrity. The aim of the study is to investigate whether ^{1}H-MRS markers will be correlated with CSF biomarkers and structural morphometry to better understand the pathologic basis of AD changes.

Description of study

The proposed analysis will be conducted on data from N = 34 cognitively normal elderly individuals, taken from the parent Alzheimer's Disease Research Center pilot study. Data collection was completed in 2020. Eligibility criteria included age of 50-95 (inclusive) years, and evidence of normal cognition determined by the following clinical assessments: Clinical Dementia Rating (CDR) of 0, Geriatric Depression Scale (GDS) score ≤ 2, Brief Cognitive Rating Scale (BCRS) score ≤ 2, and Mini Mental Status Examination (MMSE) score ≥ 27. Exclusion criteria included any objective evidence for cognitive decline or functional impairment of daily living, contraindications to MRI, diagnosis of any brain disease or MRI evidence of brain damage, significant history of alcoholism or drug abuse, history of psychiatric illness, and/or evidence of uncontrolled hypertension, cardiac, pulmonary, vascular, metabolic, or hematological conditions.

Hypotheses

Aim1:

To examine the relationship between regional metabolite levels and neurodegeneration.

Hypotheses1:

- NAA measured from the hippocampus will directly correlate with hippocampal volume.
- NAA measured from the precuneus will directly correlate with precuneus cortical thickness.
- NAA measured from the posterior cingulate will directly correlate with posterior cortical thickness.
- No statistically significant correlations will be observed between any metabolite in the lateral occipital gyrus, and the lateral occipital gyrus cortical thickness.

Aim2:

To examine the relationship between regional metabolite levels and tau pathology.

Hypotheses2:

- NAA measured from the hippocampus, precuneus, and posterior cingulate will indirectly correlate CSF p-tau.
- No statistically significant correlations will be observed between any metabolite in the lateral occipital gyrus and CSF p-tau.

Aim3:

To examine whether APOE4 risk status mediates the associations between regional metabolite levels and tau pathology.

Hypotheses3:

- APOE4 carriers will have lower mean NAA in all regions except for the lateral occipital gyrus (negative control) compared to APOE4 non-carriers.
- Statistically significant correlations will be observed between NAA in all regions except for the lateral occipital gyrus, and p-tau.

Methods

Measures

The exposures of the study include 5 metabolites concentrations values measured from 4 regions, in mM units. The 5 metabolites are NAA, CHO, CR, MINO, and GLX, and the 4 regions are hippocampus, precuneus, posterior cingulate, and lateral occipital gyrus in brain. The outcomes are (1) morphometry measures of corresponding regions, which are hippocampal volume, precuneus cortical thickness, posterior cortical thickness, and lateral occipital gyrus cortical thickness; (2) CSF p-tau. The covariates incorporated into the study include the continuous variable, age, and categorical variables ApoE status and sex.

Statistical Analyses

Descriptive analysis

All statistical analyses were performed using R Software. Conduct the univariate analysis for demographical variables including age, gender, Months between CSF to MRI, and ptau181. Also, boxplot was used to visualize the regional 5 metabolites concentrations.

Correlation

Pearson correlation was performed to find the association between 5 metabolites measured from 4 regions and (1) morphometry measures of corresponding regions; (2)p-tau. Three kinds of Pearson correlation models were conducted, which are crude, adjusted by age, as well as adjusted by age and gender. For the significant correlated metabolites and morphometry measures or p-tau, linear regression model was used to visualize the data, and Jackknife procedure was used to test the accuracy of the correlation.

Comparison

To investigate whether ApoE4_carrier impact the 5 metabolites from 4 regions adjusted by age, one-way ANCOVA was performed, and boxplot was conducted to visualize the significant findings.

Subgroup Analysis

Pearson correlation (three models) was also used to do subgroup analysis by APOE-carrier (the correlation between metabolites and p-tau).

Multiple Imputation

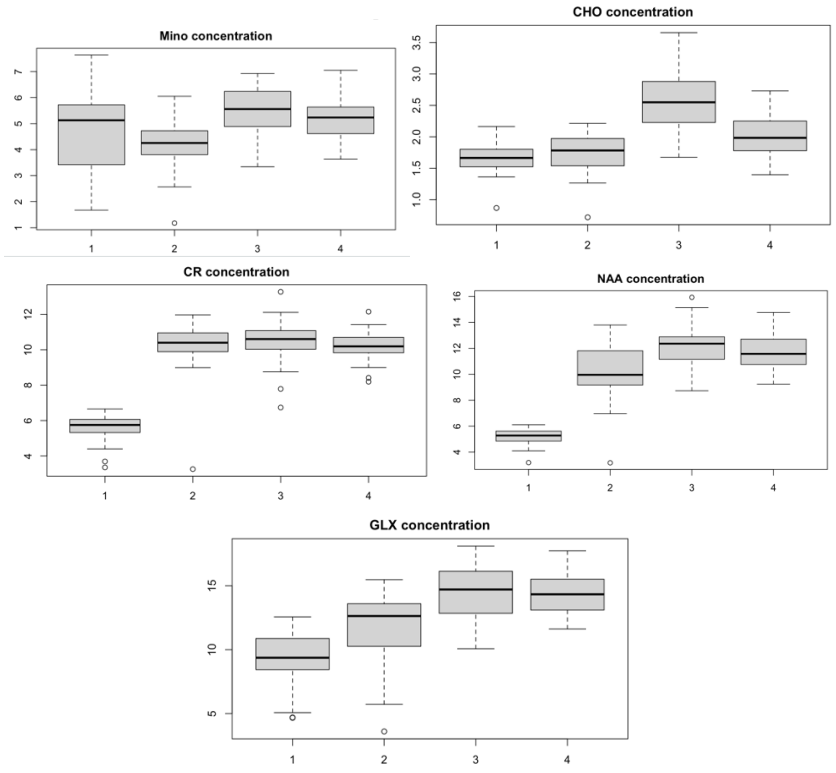
Multiple imputation will be used to replace missing data in p-tau. Then using the imputed dataset, correlation will be calculated to compare with the results gained from non-missing p-tau.

Results

Descriptive analysis

Characteristic of demographic variables

	N=34
Age (Med [IQR])	68.00 [62.25;76.00]
Gender (count (%))	
Male	12 (35.30)
Female	22 (65.00)
Months between CSF to MRI (Med [IQR])	38.00[6.00;45.00]
ptau181(Med [IQR])	34.26[29.68;42.57]



Boxplots of distributions of 5 metabolites in 4 regions.

Advanced Analysis

1. Correlation between regional metabolite levels and neurodegeneration

Table1a. Correlation between 5 metabolites concentrations and **Hippocampus volume.**

	Adjusted by age, gender		Adjusted by age		Crude	
	estimate	p-value	estimate	p-value	estimate	p-value
MINO	-0.096	0.608	-0.066	0.719	-0.111	0.539
CHO	0.010	0.959	0.033	0.858	-0.039	0.830
CR	-0.028	0.882	0.021	0.906	0.113	0.531
NAA	0.283	0.123	0.295	0.101	0.405	0.019
GLX	0.115	0.537	0.142	0.438	0.225	0.208

* 5 metabolites concentrations measured from the L hippocampus, in mM units.
* Left-Hippocampus/eTIV
* 33 observations

Table 1b. Jackknife sensitivity analysis for the significant data. (NAA in left hippocampus and Left-Hippocampus/eTIV)

	estimate	p-value	Jackknife sensitivity analysis
NAA (crude)	0.405	0.019	33/33

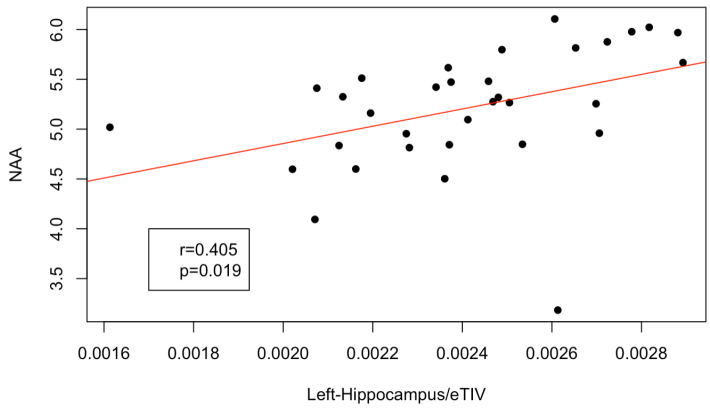


Fig. 1. Association between NAA in left hippocampus and Hippocampus volume. r, crude correlation coefficient value; p, p value.

Table2. Correlation between 5 metabolites concentrations and **lateral occipital gyrus cortical thickness.**

	Adjusted by age, gender		Adjusted by age		Crude	
	estimate	p-value	estimate	p-value	estimate	p-value
MINO	-0.050	0.787	-0.031	0.862	-0.012	0.947
CHO	0.030	0.870	0.053	0.771	0.045	0.802
CR	0.100	0.587	0.168	0.351	0.169	0.340
NAA	0.243	0.181	0.310	0.079	0.316	0.068
GLX	0.183	0.315	0.251	0.159	0.259	0.139

* 5 metabolite concentrations measured from the lateral occipital gyrus, in mM units.
* Lateraloccipital_thickavg = (L_lateraloccipital_thickavg+R_lateraloccipital_thickavg)/2
* GLX=GLU+GLN
* 34 observations

Table3a. Correlation between 5 metabolites concentrations and **precuneus cortical thickness.**

	Adjusted by age, gender		Adjusted by age		Crude	
	estimate	p-value	estimate	p-value	estimate	p-value
MINO	0.215	0.253	0.220	0.235	0.233	0.200
CHO	0.151	0.425	0.154	0.407	0.139	0.447
CR	0.367	0.046	0.363	0.045	0.335	0.061
NAA	0.287	0.124	0.286	0.119	0.290	0.107
GLX	0.231	0.219	0.230	0.212	0.198	0.278

* 5 metabolite concentrations measured from the precuneus, in mM units.
* Precuneus_thickavg = (L_precuneus _thickavg+R_precuneus _thickavg)/2
* GLX=GLU+GLN
* 32 observations

Table 3b. Jackknife sensitivity analysis for the significant data. (CR in precuneus and precuneus cortical thickness)

	estimate	p-value	Jackknife sensitivity analysis

CR (Adjusted by age, gender)	0.367	0.046	15/32
CR (Adjusted by age)	0.363	0.045	17/32

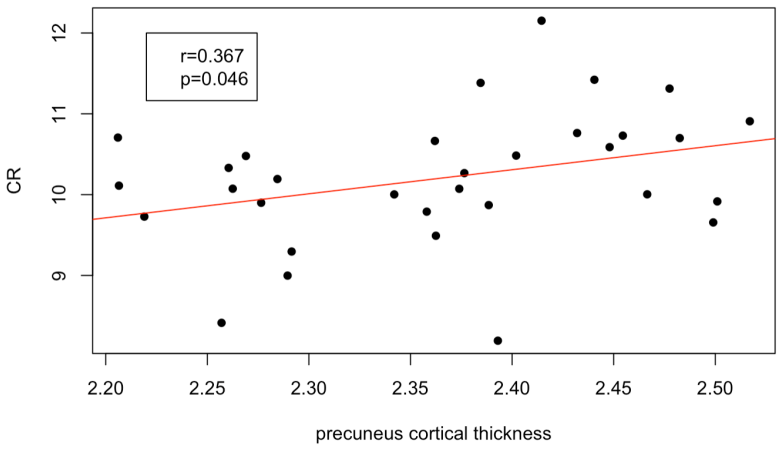


Fig. 2. Association between NAA in precuneus and precuneus cortical thickness. r, age and gender adjusted correlation coefficient value, p, p value.

Table4. Correlation between 5 metabolites concentrations and **posterior cingulate cortical thickness.**

	Adjusted by age, gender		Adjusted by age		Crude	
	estimate	p-value	estimate	p-value	estimate	p-value
MINO	0.191	0.360	0.053	0.797	0.054	0.791
CHO	0.075	0.722	0.008	0.970	0.011	0.957
CR	0.201	0.336	0.045	0.825	0.067	0.738
NAA	0.194	0.353	0.090	0.661	0.103	0.610
GLX	0.393	0.052	0.229	0.261	0.240	0.228

* 5 metabolite concentrations measured from the posterior cingulate, in mM units.
* Posteriorcingulate_thickavg = (L_ posteriorcingulate _thickavg+R_ posteriorcingulate _thickavg)/2
* GLX=GLU+GLN
* 27 observations

2. Relationship between regional metabolite levels and tau pathology

Table 5a. Correlation between 5 metabolites concentrations from 4 regions and ptau

		Adjusted by age, gender		Adjusted by age		Crude	
		estimate	p-value	estimate	p-value	estimate	p-value
Lateral occipital (N=24)	MINO	-0.066	0.771	-0.036	0.872	-0.064	0.765
	CHO	-0.217	0.331	-0.171	0.436	-0.154	0.472
	CR	-0.054	0.813	0.062	0.779	0.042	0.847
	NAA	0.041	0.857	0.159	0.468	0.116	0.588
	GLX	0.070	0.756	0.163	0.458	0.110	0.609
Precuneus (N=22)	MINO	-0.081	0.734	-0.059	0.799	-0.062	0.784
	CHO	-0.190	0.421	-0.176	0.446	-0.146	0.517
	CR	-0.123	0.607	-0.040	0.864	-0.007	0.975
	NAA	0.062	0.796	0.137	0.555	0.131	0.560
	GLX	0.090	0.707	0.152	0.510	0.173	0.441
Hippocampus (N=23)	MINO	-0.099	0.670	0.009	0.968	0.013	0.952
	CHO	-0.096	0.678	0.015	0.948	0.017	0.940
	CR	0.120	0.604	0.289	0.192	0.278	0.199
	NAA	0.243	0.288	0.308	0.163	0.282	0.192
	GLX	0.439	0.047	0.522	0.013	0.498	0.016
Posterior cingulate (N=17)	MINO	-0.064	0.820	0.091	0.736	0.099	0.706
	CHO	-0.207	0.460	-0.151	0.577	-0.158	0.544
	CR	-0.156	0.578	-0.040	0.882	-0.068	0.796
	NAA	0.003	0.991	0.029	0.916	0.021	0.937
	GLX	0.071	0.802	0.148	0.585	0.118	0.652

Table 5b. Jackknife sensitivity analysis for the significant data. (GLX in hippocampus and ptau)

	estimate	p-value	Jackknife sensitivity analysis

GLX (Adjusted by age, gender)	0.439	0.047	8/23
GLX (Adjusted by age)	0.522	0.013	23/23
GLX (Crude)	0.498	0.016	22/23

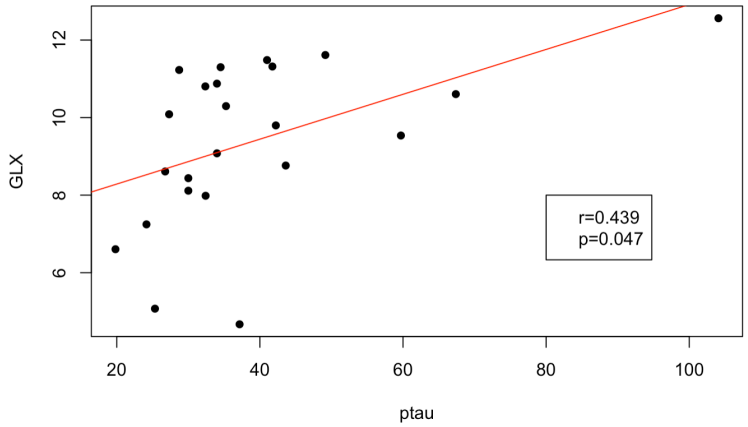


Fig. 3. Association between GLX in hippocampus and ptau. r, age and gender adjusted correlation coefficient value, p, p value.

3. whether APOE4 risk status mediates the associations between regional metabolite levels and tau pathology.

Table 6a. Comparison of metabolite concentrations measured from the **L hippocampus** stratified by ApoE4_carrier. (**Adjusted by age**)

	F value	p-value
MINO	0.069	0.794
CHO	0.094	0.762
CR	2.102	0.157
NAA	0.248	0.622
GLX	3.416	0.074

* 5 metabolite concentrations measured from the L hippocampus, in mM units.
* One-way ANCOVA test

Table 6b. Comparison of metabolite concentrations measured from the **precuneus** stratified by ApoE4_carrier. (**Adjusted by age**)

	F value	p-value

MINO	2.132	0.155
CHO	2.127	0.156
CR	0.289	0.595
NAA	0.885	0.355
GLX	0.148	0.703

* 5 metabolite concentrations measured from the precuneus, in mM units.
* One-way ANCOVA test

Table 6c. Comparison of metabolite concentrations measured from the **lateraloccipital** stratified by ApoE4_carrier. (**Adjusted by age**)

	F value	p-value
MINO	1.617	0.213
CHO	0.476	0.495
CR	0.044	0.836
NAA	0.570	0.456
GLX	0.238	0.629

* 5 metabolite concentrations measured from the lateral occipital gyrus, in mM units.
* One-way ANCOVA test

Table 6d. Comparison of metabolite concentrations measured from the **posterior cingulate** stratified by ApoE4_carrier. (**Adjusted by age**)

	F value	p-value
MINO	1.040	0.318
CHO	1.120	0.300
CR	0.002	0.965
NAA	0.077	0.784
GLX	4.298	0.049

* 5 metabolite concentrations measured from the posterior cingulate, in mM units.
* One-way ANCOVA test

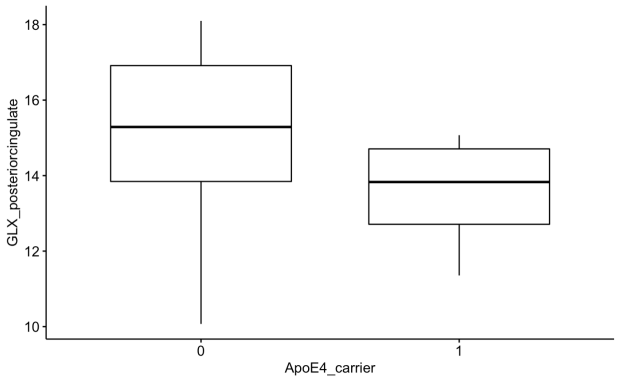


Fig 4. Boxplot for GLX in posterior cingulate stratified by ApoE4_carrier.

Table 7a. Subgroup analysis for the correlation between metabolites and ptau by APOE4-carrier. (APOE4-carrier=0)

		Adjusted by age, gender		Adjusted by age		Crude	
		estimate	p-value	estimate	p-value	estimate	p-value
Lateral occipital (N=17)	MINO	-0.052	0.853	-0.004	0.988	-0.179	0.491
	CHO	-0.093	0.743	-0.006	0.984	0.028	0.915
	CR	0.129	0.648	0.262	0.327	0.124	0.635
	NAA	-0.030	0.915	0.191	0.480	0.065	0.804
	GLX	0.033	0.906	0.198	0.463	-0.001	0.997
Precuneus (N=16)	MINO	-0.194	0.506	-0.153	0.586	-0.116	0.668
	CHO	-0.373	0.189	-0.277	0.318	-0.097	0.720
	CR	-0.190	0.515	-0.075	0.791	0.034	0.899
	NAA	-0.278	0.336	-0.102	0.719	-0.075	0.784
	GLX	-0.321	0.263	-0.233	0.404	-0.062	0.819
Hippocampus (N=16)	MINO	-0.120	0.682	0.065	0.818	0.148	0.584
	CHO	-0.113	0.701	0.084	0.766	0.144	0.595
	CR	0.375	0.186	0.568	0.027	0.455	0.077
	NAA	0.214	0.462	0.387	0.154	0.247	0.356

	GLX	0.332	0.246	0.487	0.065	0.389	0.137
Posterior cingulate (N=11)	MINO	-0.207	0.593	0.234	0.515	0.210	0.536
	CHO	-0.483	0.1877	-0.040	0.913	-0.044	0.897
	CR	-0.127	0.745	0.250	0.486	0.269	0.424
	NAA	-0.297	0.438	-0.009	0.981	-0.005	0.988
	GLX	-0.230	0.551	0.054	0.881	0.107	0.755

Table 7b. Subgroup analysis for the correlation between metabolites and ptau by APOE-carrier. (APOE4-carrier=1)

		Adjusted by age, gender		Adjusted by age		Crude	
		estimate	p-value	estimate	p-value	estimate	p-value
Lateral occipital (N=7)	MINO	0.441	0.457	0.475	0.341	0.335	0.463
	CHO	-0.430	0.469	-0.460	0.359	-0.458	0.301
	CR	-0.065	0.918	-0.092	0.863	-0.205	0.659
	NAA	0.218	0.725	0.065	0.903	0.115	0.805
	GLX	0.557	0.329	0.329	0.593	0.206	0.657
Precuneus (N=6)	MINO	-0.111	0.889	0.099	0.874	0.316	0.541
	CHO	-0.156	0.844	-0.280	0.648	-0.065	0.902
	CR	-0.047	0.953	-0.101	0.872	-0.366	0.475
	NAA	0.446	0.554	0.291	0.635	0.372	0.468
	GLX	0.926	0.074	0.761	0.135	0.796	0.058
Hippocampus (N=7)	MINO	-0.031	0.960	0.023	0.966	-0.060	0.898
	CHO	-0.725	0.165	-0.741	0.092	-0.673	0.098
	CR	-0.217	0.726	-0.122	0.818	-0.128	0.785
	NAA	0.482	0.411	0.327	0.527	0.375	0.408

	GLX	0.782	0.118	0.763	0.077	0.765	0.045
Posterior cingulate (N=6)	MINO	0.167	0.833	0.209	0.736	0.218	0.678
	CHO	-0.085	0.915	-0.165	0.790	-0.297	0.568
	CR	-0.938	0.062	-0.866	0.057	-0.732	0.098
	NAA	0.308	0.692	0.168	0.787	0.052	0.922
	GLX	0.416	0.584	0.436	0.463	0.501	0.312

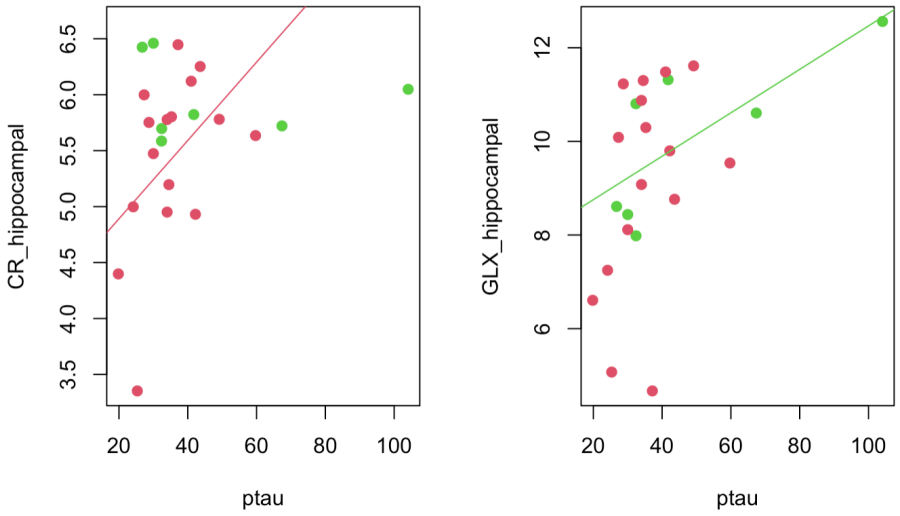
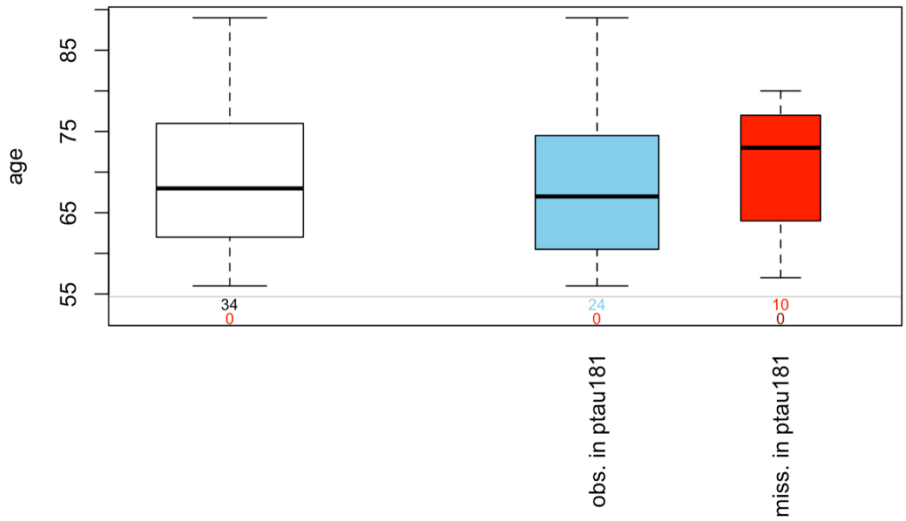


Fig 5a (left). Relationship between ptau and CR measured from hippocampus. Red point, APOE4-carrier=0; green point, APOE4-carrier=1. Red line shows the linear regression between ptau and CR measured from hippocampus among the patients without APOE4-carrier.

Fig 5b (right). Relationship between ptau and GLX measured from hippocampus. Red point, APOE4-carrier=0; green point, APOE4-carrier=1. Green line shows the linear regression between ptau and GLX measured from hippocampus among the patients with APOE4-carrier.

4. Multiple Imputation



ptau181:
0 represents the value of ptau181 is missing
1 represents the value of ptau181 is not missing

	ptau181		p
	0	1	
n	10	24	
NAA_lateraloccipital (median [IQR])	10.53 [8.91, 11.64]	10.27 [9.26, 11.64]	0.88
NAA_precuneus (median [IQR])	13.60 [12.74, 14.19]	13.55 [12.37, 14.90]	0.791
NAA_hippocampal (median [IQR])	5.13 [4.87, 5.32]	5.41 [4.90, 5.81]	0.21
NAA_posteriorcingulate (median [IQR])	13.57 [13.10, 14.42]	13.33 [12.64, 15.17]	0.88
CHO_lateraloccipital (median [IQR])	1.68 [1.43, 1.97]	1.76 [1.48, 2.03]	0.496
CHO_precuneus (median [IQR])	1.87 [1.79, 2.20]	2.09 [1.91, 2.37]	0.241
CHO_hippocampal (median [IQR])	1.52 [1.51, 1.73]	1.67 [1.57, 1.80]	0.308
CHO_posteriorcingulate (median [IQR])	2.53 [2.36, 3.02]	2.77 [2.61, 3.03]	0.241
CR_lateraloccipital (median [IQR])	11.15 [10.77, 11.44]	11.01 [9.91, 11.99]	0.734
CR_precuneus (median [IQR])	11.87 [11.50, 12.31]	12.56 [11.78, 12.71]	0.273
CR_hippocampal (median [IQR])	5.76 [5.36, 6.10]	5.75 [5.33, 6.02]	0.875
CR_posteriorcingulate (median [IQR])	12.34 [11.59, 13.17]	12.46 [11.82, 12.89]	0.94
MINO_lateraloccipital (median [IQR])	4.56 [3.94, 4.91]	4.49 [4.08, 5.50]	0.427
MINO_precuneus (median [IQR])	5.78 [4.84, 5.85]	6.17 [5.40, 6.47]	0.29
MINO_hippocampal (median [IQR])	4.63 [3.42, 5.29]	5.20 [3.47, 5.88]	0.557
MINO_posteriorcingulate (median [IQR])	6.85 [5.59, 7.38]	6.48 [5.74, 7.06]	0.734
GLX_lateraloccipital (median [IQR])	13.27 [11.54, 14.73]	13.45 [11.24, 14.57]	0.85
GLX_precuneus (median [IQR])	16.91 [15.55, 18.20]	17.65 [16.38, 18.74]	0.364
GLX_hippocampal (median [IQR])	9.24 [8.67, 9.90]	9.80 [8.28, 11.05]	0.754
GLX_posteriorcingulate (median [IQR])	16.99 [16.19, 19.18]	17.83 [16.56, 19.04]	0.678

All the P values are greater than 0.05, which means that the metabolites values are not significant different due to the ptau181 is missing or not. Thus, the next step, we can use the non-missing ptau181 to investigate the association between the values of Cho, Cr, Glx, mino and NAA from left hippocampus and ptau181.

	Adjusted by age, gender		Adjusted by age		Crude	
	estimate	p-value	estimate	p-value	estimate	p-value
MINO_L	-0.098	0.663	-0.0797	0.717	-0.082	0.700
CHO_L	-0.208	0.351	-0.185	0.396	-0.140	0.513
CR_L	-0.080	0.722	0.016	0.939	0.037	0.860
NAA_L	0.051	0.819	0.148	0.498	0.133	0.534
GLX_L	0.175	0.435	0.232	0.287	0.190	0.373

The output shows that all of the correlation between the values of Cho, Cr, Glx, mino and NAA from left hippocampus and ptau181 are non-significant. However, we can still use multiple imputation to check whether the metabolites values would be significant if the ptau181 non-missing.

	1 <dbl>	2 <dbl>	3 <dbl>	4 <dbl>	5 <dbl>
10	28.72137	35.26627	28.72137	19.81896	34.51121
13	41.74783	30.00000	41.00000	30.00000	32.41694
14	41.00000	34.51121	34.00000	59.70750	25.33774
15	24.15024	19.81896	25.33774	30.00000	26.76535
20	32.37640	43.61227	37.16239	30.00000	26.76535
25	67.38036	43.61227	30.00000	19.81896	41.74783
26	32.41694	19.81896	32.41694	59.70750	26.76535
28	26.76535	67.38036	41.00000	30.00000	27.31488
29	26.76535	41.74783	41.00000	30.00000	67.38036
32	41.00000	34.00000	35.26627	67.35108	67.38036

Fig 6. An example of the lists for imputed missing ptau181 values

Table 8. Correlation between 5 metabolites concentrations measured from left hippocampus and imputed ptau

	Adjusted by age, gender		Adjusted by age		Crude	
	estimate	p-value	estimate	p-value	estimate	p-value

MINO_L	-0.050	0.802	0.034	0.859	0.036	0.849
CHO_L	-0.055	0.777	0.024	0.897	0.027	0.887
CR_L	0.091	0.637	0.218	0.256	0.209	0.280
NAA_L	0.199	0.282	0.261	0.159	0.236	0.208
GLX_L	0.316	0.115	0.046	0.059	0.380	0.056

Interpretation

1. Relationship between regional metabolite levels and neurodegeneration.

According to the results shown in the table 1a, table 2, table 3a and table 4, we can find that
(1) only NAA measured from left hippocampus is significantly correlate with hippocampus volume,
(2) there are no statistically significant correlations between 5 metabolites measured from lateral occipital gyrus and the lateral occipital gyrus cortical thickness,
(3) only CR measured from precuneus is significantly correlate with precuneus cortical thickness,
(4) and there are no statistically significant correlations between 5 metabolites measured from posterior cingulate and the posterior cortical thickness.

For the two significant correlation, jackknife sensitivity analysis results shown in table 1b and table 3b, we can find that:
(1) for the NAA- hippocampus volume correlation, the accuracy is 33/33, which means that the significant correlation is highly reliable,
(2) for the CR- precuneus cortical thickness correlation, the accuracy for model 1 (adjusted by age and gender) is 15/32, the accuracy for model 2 (adjusted by age) is 17/32, which means that the significant correlation is not that reliable.

The hypothesis assumed that, first, NAA measure from hippocampus, precuneus and posterior cingulate will directly correlate with their corresponding morphometry measures. However, we only find that NAA measure from hippocampus directly correlate with hippocampus volume. Thus, for

precuneus and posterior cingulate, the hypothesis cannot be confirmed. What's more, the hypothesis also assumed that no statistically significant correlations will be observed between any metabolite in the lateral occipital gyrus, and the lateral occipital gyrus cortical thickness. This hypothesis can be confirmed by the results shown in table 2.

2. Relationship between regional metabolite levels and tau pathology.

According to the results shown in table 5a, GLX measured from hippocampus significantly correlated with p-tau in all three models. There are no statistically significant correlations between any metabolites measured from lateral occipital gyrus, precuneus and posterior cingulate and p-tau.

The jackknife sensitivity analysis results for GLX-p-tau correlation is shown in table 5b, the accuracy for model 1 (adjusted by age and gender) is 8/23, the accuracy for model 2 (adjusted by age) is 23/23, and the accuracy for model 3 (crude) is 22/23, which means that the significant correlations in model 2 and crude model are highly reliable.

The hypothesis related to the p-tau correlation assumed that NAA measured from the hippocampus, precuneus, and posterior cingulate will indirectly correlate CSF p-tau. However, we found that there is no relationship between NAA measured from any regions and p-tau. What's more, the hypothesis also assumed that no statistically significant correlations will be observed between any metabolite in the lateral occipital gyrus and CSF p-tau. The later hypothesis can be sure based on the results we found in table 5a.

3. whether APOE4 risk status mediates the associations between regional metabolite levels and tau pathology.

Comparison

Table 6a, 6b, 6c and 6d shows the results of comparative analysis of metabolite concentrations measured from 4 regions stratified by ApoE4_carrier using one-way ANCOVA methods. The results show that after adjusting by the covariate, age, there is no difference of all 5 metabolites in hippocampus, precuneus, and lateral occipital gyrus stratified by ApoE4. Only GLX measured from posterior cingulate with ApoE4 are significantly different with GLX

without ApoE4. The boxplot (Fig 4) shows that in posterior cingulate, the participants ApoE4 have significantly lower GLX concentration than whom without ApoE4.

Subgroup Analysis

Table 7a and 7b show the subgroup analysis results for the correlation between metabolites and ptau by APOE4. For the APOE4 non carriers, only CR measured from hippocampus is significantly correlated with p-tau. For the APOE4 carriers, only GLX measured from hippocampus is significantly correlated with p-tau.

The hypothesis assumed that APOE4 carriers will have lower mean NAA in all regions except for the lateral occipital gyrus (negative control) compared to APOE4 non-carriers. However, APOE4 carriers only significantly lower the mean GLX in hippocampus. In addition, NAA of AOPE4 carriers in all regions have no difference with APOE4 non-carriers. The hypothesis assumed that statistically significant correlations will be observed between NAA in all regions except for the lateral occipital gyrus, and p-tau, however, for the both two subgroups, NAA in any regions are not correlated with p-tau.

4. Multiple Imputation

We used package mice to perform multiple imputation and imputed the missing values of variable ptau181. Age, gender, race is selected as covariates to generate missing ptau181 values. Fig 6 is an example of the lists for imputed missing ptau181 values. In this case, we have imputed ptau181 for fifty times, and we were only generating the ptau181 missing values (with the function block in R) even though we were using other variables to perform the imputation. After we obtained the imputed dataset, we used a function from package miceadds called micombine.cor to calculate the correlation association between the values of Cho, Cr, Glx, mino and NAA from left hippocampus and ptau181. This function would automatically provide the final correlation estimates and p values with the imputed ptau181 values that was generated for fifty time. Table 8 is the output of the correlation between 5 metabolites and ptau181.

According to the output shown in table 8, although some of the p values reduced comparing to the output from non-missing ptau181 values, the correlation between the values of Cho, Cr, Glx, mino and NAA from left hippocampus and ptau181 are still non-significant, which was very similar to the

result of the correlation output from non-missing ptau181 values. This is probably due to the fact that our sample size is too small(N=34). Therefore, we eventually decided to perform our analysis with non-missing ptau181 values.

Discussion

Conclusion

Correlation:

(1) NAA measured from hippocampus directly correlate with Hippocampus volume
CR measured from precuneus directly correlate with precuneus cortical thickness
(2) GLX measured from the hippocampus indirectly correlate with CSF p-tau.

Comparison

APOE4 carriers have lower mean GLX in hippocampus compared to APOE4 non-carriers.

Subgroup analysis

Without APOE-carrier: CR measured from the hippocampus indirectly correlate with CSF p-tau.
With APOE-carrier: GLX measured from the hippocampus indirectly correlate with CSF p-tau.

Limitation

(1) Small sample size: we only have a total number of 34 participants, so the sample size is small, and may cause bias.
(2) Missing values: except p-tau have missing values, some other variables have missing values, too. Thus, it is easy to

Next Step

(1) Multiple imputation: in the study, multiple imputation only used to find the correlation metabolites measured from hippocampus and imputed p-tau. In the next step, we can still use the multiple imputation to examine the correlation metabolites measured from other three regions and imputed p-tau.

(2) Bonferroni correction: In the next step, we are planning to use Bonferroni correction to adjust the p value of multiple tests.