Supplementary Online Content

Bustillo JR, Chen H, Jones T, et al. Increased glutamine in patients undergoing long-term treatment for schizophrenia: a proton magnetic resonance spectroscopy study at 3 T. *JAMA Psychiatry*. Published online January 8, 2014. doi:10.1001/jamapsychiatry.2013.3939

This supplementary material has been provided by the authors to give readers additional information about their work.

A- Methods: Partial Volume Correction

The results from LCModel were corrected for CSF, WM, and GM content (partial volume effects) as previously reported[1]. Briefly, tissue segmentation was performed on the T_1-weighted image using SPM5. The individual GM, WM, and CSF maps are then registered to the spectroscopic region of interest. To obtain the fractional GM, WM, and CSF in the MRS voxel, the pixel values of the maps are summed and normalized over the volume of voxel for each tissue class. Finally, the LCModel results are corrected for partial volume effects in each voxel according to the equation:

$$[M] = \frac{[M]_{LCM} \times (f_{GM} \times R_{H2O\_GM} + f_{WM} \times R_{H2O\_WM} + f_{CSF} \times R_{H2O\_CSF})}{(1 - f_{CSF}) \times R_M} \qquad [1]$$

where $[M]_{LCM}$ is concentration in mmoles per Kg of MR-visible water (mmolal) determined by LCmodel, based on using tissue water as a concentration reference ; f_{GM}, f_{WM}, and f_{CSF} are the water density fractions for GM, WM, and CSF, respectively; and the R_{H2O} terms are the relaxation attenuation factors for the water signal in each tissue class, based on reported values for T_1 and T_2 and the equation $R_{H2O\_y} = \exp[-TE/T_{2\_H2O\_y}](1-\exp[-TR/T_{1\_H2O\_y}])$, where $T_{1\_H2O\_y}$ and $T_{2\_H2O\_y}$ are the T_1 and T_2 relaxations times of water in compartment y, TE is the sequence echo time, and TR is the repetition time. Similarly, R_M is the relaxation attenuation factor for the metabolite signal, assumed to be similar in both GM and WM.

The fractional water densities appearing in Eq. [1], are related to the tissue volume fractions obtained by tissue segmentation by taking into account the relative water densities (WD) in each volume fraction:

$$f_x = \frac{f_{x\_vol} \times WD_x}{f_{GM\_vol} \times WD_{GM} + f_{WM\_vol} \times WD_{WM} + f_{CSF\_vol} \times WD_{CSF}} \qquad [2]$$

where the various terms refer to the volume fractions and associated water densities of each tissue or CSF (i.e., x = GM, WM, or CSF). In the present study, we used a CSF T_1 value of 4 s based on a recent report [2] and a CSF T_2 estimate of 2.47 s based on a previous measurement at our site. Otherwise, the previously reported T_1, T_2, and WD values used were as follows: GM: T_1=1.304 s, T_2=0.093 s [3], WD=0.78 [4]; WM: T_1=0.660 s, T_2=0.073 s [3]; WD=0.65 [4]; CSF: WD=0.97 [4,5]. Estimates of metabolite T_1 and T_2 values at 3T were drawn from Mlynarik et al. [6]. The Gln T_1 and T_2 values were assumed to be equal to the Glu values.

B- eTable 1. Demographic and clinical characteristics of the subject sample.

	Schizophrenia (n=84)	***Controls*** (n=81))
	mean (SD)	mean (SD)
Age (years)	36.7(13.9)	35.2 (11.8)
	range: 16-64	range: 17-65
Gender (male/female)	71/13	54/27*
Occupation	5.3 (1.6)	3.9 (1.2)*
Parental Occupation	4.1(1.7)	4.2 (1.7)
Smoker (yes/no)	32/46	19/61*
MATRICS Overall T score	31.1 (13.2)	49.8 (9.7)*
Age Onset Psychosis years	20.8 (8.4)	N/A
Positive symptoms	14.5 (5.6)	N/A
Negative Symptoms	15.1 (5.6)	N/A
Tardive dyskinesia	1.5 (0.7)	N/A
Akathisia	0.18 (0.52)	N/A
Parkinsonism	9.5 (2.1)	N/A
Antipsychotic dose (OLZ equivalents)	19.8 (34.8)	N/A
Clozapine (yes/no)	10/74	
*Glutamatergic drug^*	11/73	
Antipsychotic (yes/no)	81/3	
Antipsychotic 1st/2nd generation	12/69	
Mood stabilizer (yes/no)	4/80	
Antidepressant (yes/no)	24/60	
Benzodiazepine (yes/no)	19/65	
Anticholinergic (yes/no)	13/71	
Beta-blocker (yes/no)	7/77	

*p<0.05

^clozapine and valproate

C- eTable 2. Measures of metabolites fit (Cramer-Rao Lower Bounds- CRLB), spectral quality (Full Width Half Max and Signal to Noise ratio) and voxel tissue composition

	Schizophrenia (n=84)	***Controls*** (n=81)
	mean (SD) range	mean (SD) range
*Glutamine CRLB^*	19.6 (5.1) 12-30	18.9 (4.0) 12-29
Glutamate CRLB	8.7 (2.3) 5-17	7.3* (1.5) 5-13
NAAc CRLB	3.0 (0.8) 2-6	2.5* (0.5) 2-3
Choline CRLB	2.9 (0.5) 2-4	2.8 (0.4) 2-4
Myo-inositol CRLB	5.6 (1.2) 3-12	5.0* (0.6) 4-7
Total-creatine CRLB	2.1(0.3) 2-3	2.0* (0.1) 2-3
Full Width Half Max (FWHM)	0.05 (0.01) 0.03-0.1	0.04* (0.01) 0.03-0.09
Signal to Noise ratio (SNR)	36.3 (7.6) 19-51	42.2* (6.8) 26-62
Gray matter %	0.58 (0.06) 0.42-0.73	0.62* (0.04) 0.53-0.72
White matter %	0.18 (0.04) 0.09-0.29	0.18 (0.03) 0.09-0.27
Cerebro Spinal Fluid %	0.24 (0.07) 0.14-0.45	0.20* (0.05) 0.12-0.34
**p<0.05*		
^Schizophrenia n=72, Controls n=76		

Cramer Rao Lower bounds (CRLB), not FWHM or SNR, is the preferred measure of spectral quality and fit[7,8]. After co-varying for NAAc CRLB, group differences in NAAc were no longer significant [F(1, 161)=0.15, p=0.7].

The effect of differences in gray matter and cerebrospinal fluid voxel composition among groups is accounted for with partial volume correction (see Supplementary materials A).

D. Effects of psychotropic medications on metabolite group differences

D. i. eTable 3.

Metabolite	Psychotropic	Drug x Age (in schizophrenia)	Drug (in schizophrenia)	Group differences^ (adjusting for Age)
Glutamine	Clozapine	F(1,68)=6.4, p=0.01*	N/A	F(1,135)=3.5, p=0.06
	Glutamatergic	F(1,68)=6.4, p=0.01	N/A	F(1,134)=3.3, p=0.07
	First generation antipsychotic	p=0.9	p=0.9	N/A
	No antipsychotic	p=0.8	p=0.9	N/A
	Mood stabilizer	p=0.2	F(1,69)=3.5, p=0.07[@]	F(1,141)=4.6, p=0.06
	Antidepressant	p=0.2	p=0.6	N/A
	Benzodiazepine	F(1,68)=6.7, p=0.01	N/A	F(1,129)=3.5, p<0.001
	Anticholinergic	p=0.6	p=0.9	N/A
	Betablocker	p=0.4	p=0.7	N/A
Glutamine/glutamate	Clozapine	p=0.2	p=0.2	N/A
	Glutamatergic	p=0.2	p=0.2	N/A
	First generation antipsychotic	p=0.8	p=0.9	N/A
	No antipsychotic	p=0.5	p=0.7	N/A
	Mood stabilizer	p=0.9	p=0.5	N/A
	Antidepressant	p=0.9	p=0.7	N/A
	Benzodiazepine	F(1,68)=6.7, p=0.01	N/A	F(1,129)=8.0, p=0.006
	Anticholinergic	p=0.9	p=0.7	N/A
	Betablocker	p=0.3	p=0.4	N/A
Choline	Clozapine	p=0.1	p=0.3	N/A
	Glutamatergic	p=0.2	p=0.1	N/A
	First generation antipsychotic	p=0.8	p=0.6	N/A
	No antipsychotic	p=0.5	p=0.6	N/A
	Mood stabilizer	p=0.7	p=0.8	N/A
	Antidepressant	p=0.2	F(1,81)=4.2, p=0.04	F(1,138)=16.2, p<0.001
	Benzodiazepine	p=0.4	p=0.6	N/A
	Anticholinergic	p=0.2	p=0.7	N/A
	Betablocker	p=0.9	p=0.8	N/A
		Drug x Smoking (in schizophrenia)	**Drug (in schizophrenia)**	**Group x Smoking**
NAAc	Clozapine	p=0.7	p=0.9	N/A
	Glutamatergic	p=0.4	p=0.6	N/A
	First generation antipsychotic	p=0.7	p=0.1	N/A
	No antipsychotic	N/A[#]	F(1,75)=4.1, p=0.05	F(1,151)=6.0, p=0.02
	Mood stabilizer	p=0.4	p=0.4	N/A
	Antidepressant	p=0.2	p=0.2	N/A
	Benzodiazepine	p=0.1	p=0.1	N/A
	Anticholinergic	p=0.5	p=0.7	N/A
	Betablocker	p=0.4	p=0.8	N/A

^For psychotropics with Drug interaction or Drug main effect, schizophrenia subjects on that drug were removed from the schizophrenia vs control comparison to examine whether group differences remain for that metabolite
**Three clozapine subjects appear to be driving this interaction.*
[@]Only 4 subjects on mood stabilizing drugs
[#]There were no non-smokers off antipsychotic drugs; total of 3 subjects on no antipsychotic.

D.ii. Interpretation

Hence, these exploratory analyses suggest possible medication effects on glutamine (elevation with age for those on clozapine and reduction with age for those on benzodiazepines), on Gln/Glu ratio (reduction with age for those on benzodiazepines), on choline (reduction for those on antidepressants) and on NAAc (increase for those on antipsychotics, but only 3 subjects off antipsychotics). However, the principal findings (increased glutamine, Gln/Glu ratio and choline in schizophrenia), are not solely accounted by exposure to these specific group of medications.

D. References

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5. **Ashburner J, Friston KJ. Unified segmentation. *Neuroimage.* Jul 1 2005;26(3):839-851.**
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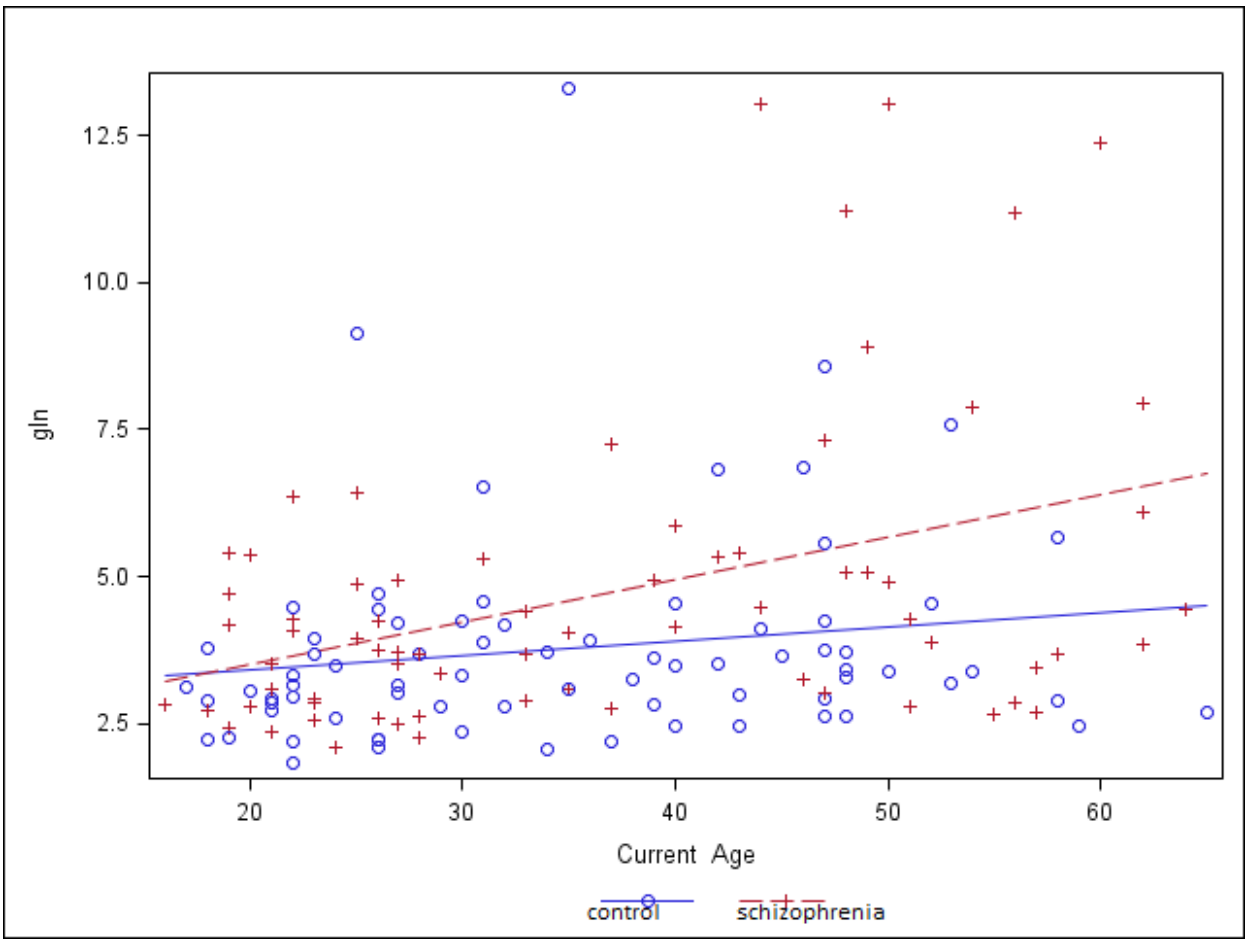
8. **Kreis R. Issues of spectral quality in clinical 1H-magnetic resonance spectroscopy and a gallery of artifacts. *NMR Biomed.* Oct 2004;17(6):361-381.**

E. Figures

eFigure 1. Marginal interaction of group by age [F(1, 144)=3.4, p=0.07] suggests a larger increment in glutamine over time in schizophrenia (0.07 mM/year) compared with controls (0.05 mM/year).



eFigure 2. Group differences in other metabolites.

Choline is increased in schizophrenia [F(1, 162)=9.94, p=0.002].