Published in final edited form as:

Schizophr Res. 2011 September ; 131(1-3): 4–10. doi:10.1016/j.schres.2011.05.024.

Magnetic resonance spectroscopy of limbic structures displays metabolite differences in young unaffected relatives of schizophrenia probands

Aristides A. Capizzano^{a,*}, Juana L. Nicoll Toscano^b, and Beng-Choon Ho^c

^aDepartment of Radiology, Division of Neuroradiology, University of Iowa Carver College of Medicine, 200 Hawkins Drive, Iowa City, IA 52242, USA

^bDepartment of Family Medicine, University of Iowa Carver College of Medicine, 200 Hawkins Drive, Iowa City, IA 52242, USA

^cDepartment of Psychiatry, University of Iowa Carver College of Medicine, 200 Hawkins Drive, Iowa City, IA 52242, USA

Abstract

Imaging studies of schizophrenia patients showed fronto-temporal brain volume deficits, while magnetic resonance spectroscopy (MRS) studies of patients and unaffected biological relatives have found a decrement of the neuronal marker N-acetyl-aspartate (NAA) in the hippocampus and frontal lobes, and increased choline-containing phospholipids. Using a 3 T MR scanner, we determined the metabolite profile within limbic regions (anterior cingulate cortex (ACC) and left hippocampus) of 36 unaffected, adolescent/young adult relatives of schizophrenia probands (first-degree=16, second-degree=20) and 25 healthy controls with no family history of schizophrenia. Significant main effects of group were found on NAA/Cho ratios for both the left hippocampus ($F=6.11$, $p=0.02$) and ACC ($F=4.89$, $p=0.03$) as well as for the left hippocampus Cho/Cr ratio ($F=5.55$, $p=0.02$). Compared to age and sex matched healthy controls without a family history of schizophrenia, first-degree relatives of probands had greater MRS metabolite deviations than second-degree relatives. Greater familial proximity to the schizophrenia proband (or higher schizophrenia susceptibility) among biological relatives was associated with stepwise lowering of NAA/Cho and elevations in Cho/Cr ratios. The observed limbic metabolite changes among young, nonpsychotic biological relatives are likely related to shared genetic vulnerability factors, and may assist in the early identification of schizophrenia for primary and secondary prevention.

© 2011 Elsevier B.V. All rights reserved.

*Corresponding author. Tel.: +1 319 384 8795; fax: +1 319 353 6275. aristides-capizzano@uiowa.edu (A.A. Capizzano), juana-nicolltoscano@uiowa.edu (J.L. Nicoll Toscano), beng-ho@uiowa.edu (B.-C. Ho).

Contributors

Author Beng-Choon Ho designed the study, recruited the subjects, performed statistical analysis and contributed to the discussion and revision of the manuscript. Author Aristides A. Capizzano set up the imaging protocol, oversaw the technical aspect of imaging and spectral acquisitions, performed the postprocessing of imaging data, generated the image database, managed the literature searches and wrote the first draft of the manuscript. Author Juana L. Nicoll Toscano performed most of the patient imaging and spectroscopy studies and collaborated with literature searches and edition of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

Keywords

Adolescence; Endophenotype; Genetics; Magnetic resonance spectroscopy; Hippocampus; Anterior cingulate cortex

1. Introduction

Schizophrenia is conceptualized as a neurodevelopmental familial disorder with a complex mode of inheritance and variable expression. Imaging studies of schizophrenia show whole brain and hippocampal volume deficits (Steen et al., 2006). Magnetic resonance spectroscopy (MRS) studies show a decrement of the neuronal marker N-acetyl-aspartate (NAA) in the hippocampus and frontal lobes in schizophrenia patients (Steen et al., 2005; Abbott and Bustillo, 2006), increased choline-containing phospholipids (O'Neill et al., 2004) and increased glutamine/glutamate (Shirayama et al., 2009). Phosphorous MRS studies have reported abnormal phospholipid metabolism in schizophrenia (Keshavan et al., 2000). Biological relatives of schizophrenia probands are at higher risk to develop schizophrenia than the general population. Furthermore, similar but less severe neuroanatomical, electrophysiological, neurocognitive and behavioral deficits have been demonstrated in relatives compared to controls without a family history of schizophrenia.

Proton magnetic resonance spectroscopy ($^1\text{HMRS}$) detects signals from relevant brain metabolites such as NAA—a putative neuronal marker (Birken and Oldendorf, 1989)—lactate (Lac), creatine (Cr), choline (Cho), myo-inositol (mIn) and glutamate plus glutamine (Glx). Abnormal $^1\text{HMRS}$ metabolite levels have been reported in a wide variety of neurologic and psychiatric disorders. Given the consistent reports of reduced brain NAA in schizophrenia (as reviewed in Steen et al., 2005), it has been hypothesized that $^1\text{HMRS}$ may also be sensitive to detect the subtle metabolite changes expected among unaffected biological relatives of schizophrenia probands. Asymptomatic subjects at genetic risk of schizophrenia display metabolic differences compared to controls: offspring of schizophrenia probands have reduced medial frontal NAA/Cho (Keshavan et al., 1997) and increased glutamate/glutamine (Tibbo et al., 2004). Significant reductions in the left hippocampus NAA/Cr (Callicott et al., 1998) and increased anterior cingulate cortex (ACC) Glx ratios (Tibbo et al., 2004) were also reported in genetic risk subjects. Also differences in Glx were detected in frontal lobe regions in schizophrenia relatives (Purdon et al., 2008; Lutkenhoff et al., 2010). On the other hand, early and late at risk state subjects, who display psychiatric symptoms, demonstrated reduced NAA and increased choline in the left frontal lobe and anterior cingulate gyrus (Jessen et al., 2006).

Such metabolic differences could potentially serve as biomarkers of disease susceptibility. Therefore, the purpose of this research is to determine the role of $^1\text{HMRS}$ metabolites in the left hippocampus and ACC as indicators of schizophrenia vulnerability. Given prior reports of reduced NAA and increased choline signal in the hippocampus and the frontal lobe of schizophrenia probands (O'Neill et al., 2004; Steen et al., 2005; Abbott and Bustillo, 2006), we hypothesized that NAA to choline signal ratios would be reduced in the left hippocampus and ACC among unaffected relatives of schizophrenia patients compared to healthy age- and sex-matched controls. Only the left hippocampus was sampled because previous studies in relatives of schizophrenia probands reported changes in the left cerebral hemisphere (Jessen et al., 2006; Purdon et al., 2008; Lutkenhoff et al., 2010). In addition, we contrasted $^1\text{HMRS}$ metabolite differences between first- and second-degree relatives of schizophrenia probands to further investigate whether $^1\text{HMRS}$ variables represent markers of familial susceptibility for the disorder. We hypothesized that the magnitude of $^1\text{HMRS}$

metabolite abnormalities would vary as a function of familial proximity to the schizophrenia proband among biological relatives.

2. Materials and methods

2.1. Subjects

The 61 subjects in this study comprised 36 relatives of schizophrenia probands (first degree=16, second degree=20) and 25 healthy normal volunteers (HNV) with no family history of schizophrenia. After complete description of the study, all subjects provided written informed consent for participation and the study was approved by the local IRB. Relatives of schizophrenia probands were recruited either through 1) schizophrenia patients who have participated in research studies or have received psychiatric treatment at the University of Iowa Health Care, or 2) advertisements in local newspapers or mental health advocacy groups. Inclusion criteria for relatives were age within the age range at-risk for developing schizophrenia (13 to 25 years), and having at least one first- or second-degree relative with schizophrenia. Family history of schizophrenia was verified using Family History-Research Diagnostic Criteria (FH-RDC) (Andreasen et al., 1977), which has well-established reliability and validity. Relatives were interviewed using the SCID-IV (Structured Clinical Interview for DSM-IV), and were excluded if they had a primary psychotic disorder (schizophrenia, schizophreniform disorder, schizoaffective disorder, or delusional disorder), or if they met criteria for substance abuse or dependence currently or within the past year. None of the relatives had schizophrenia-spectrum (schizotypal, schizoid or paranoid) personality disorders. Eighty percent of the relatives had no lifetime history of any psychiatric disorders. In the remaining 7 relatives, psychiatric diagnoses included: attention deficit hyperactivity disorder (2 first- and 1 second-degree relatives); major depressive disorder (2 second-degree relatives), depressive disorder, not otherwise specified (1 first-degree relative), and bipolar type II disorder (1 second-degree relative). HNV without a family history of schizophrenia were assessed using an abbreviated version of the Comprehensive Assessment of Symptoms and History (CASH) (Andreasen et al., 1992) to exclude subjects with current or past psychiatric illnesses and substance misuse. FH-RDC was also used to confirm the absence of a family history of schizophrenia in HNV. Additional exclusion criteria for all subjects in this study were: neurological disorders, mental retardation, unstable medical conditions or contraindications for magnetic resonance imaging (MRI).

2.2. MRI/¹HMRS studies

All imaging studies were performed in a 3 T MRI scanner (Magnetom Tim Trio; Siemens) using the manufacturer's 8 channel head coil. The MRI protocol included the following sequences: 1) coronal T1 3DMPRAGE: TR/TE/TI=2530/3.34/1100, 1 NEX, flip angle=10°, FOV=260 mm, 1.5 mm thick, voxel size=1.4×1×1.5 mm, 2) coronal T2 TSE: TR/TE=5340/14, FOV=260 mm, 3 mm thick, voxel size=1.4×1×3 mm, 3) water suppressed single voxel ¹HMRS (SVS) with PRESS volume selection centered at the left hippocampus with TR/TE=3000/30, 128 acquisitions, VOI size=4.5 cm³ and 4) water suppressed single voxel ¹HMRS with PRESS volume selection localized in the midline including the bilateral ACC with TR/TE=3000/30, 64 acquisitions, VOI size=8 cm³. Sequences 3 and 4 were repeated without water suppression with 16 acquisitions. Localized shimming was optimized at the preparation phase of sequences 3 and 4. Fig. 1a shows placement of the left hippocampus voxel and Fig. 1b shows localization of the ACC voxel.

Raw spectroscopy data files were post processed offline using the LCModel software (Provencher, 2001). The unsuppressed water signal was used for signal phasing and eddy current correction. Signals from NAA, Cr, Cho, mIn and glutamate plus glutamine (Glx)

were quantified. Choline signal is automatically corrected for the number of protons (9) by LCModel, yielding three times lower NAA/Cho ratios as when the correction is not applied since the methyl NAA and creatine groups have only 3 protons each. Spectra with full width half maximum (FWHM) over 0.1 ppm or with SNR of 5 or less as calculated by LCModel were excluded as well as fit values with Cramer Rao lower bounds (CRLB) of 20% or higher. Using this methodology, intersubject coefficients of variation for NAA of 9.5% and 5.6% were reported in HNV in the hippocampus and ACC, respectively (Capizzano et al., 2006). Fig. 2 shows LCModel output of the left hippocampus and ACC representative spectra.

2.3. Statistical analysis

The relationships between schizophrenia risk and metabolite signal ratios (i.e. NAA/Cho, NAA/Cr, Glx/Cho, Glx/Cr and Cho/Cr) were tested using ANCOVA (covariates were age and gender since both factors are known to affect MRS metabolite values) (Braun et al., 2002; Haga et al., 2009; Maudsley et al., 2009; Raininko and Mattsson, 2010). The independent measure was a dummy grouping variable in which schizophrenia risk was coded as either low (for HNV without a family history of schizophrenia), intermediate (second-degree relatives of schizophrenia probands) or high (first-degree relatives of schizophrenia probands). Next, we conducted pair-wise contrasts across the 3 comparison groups using Cohen's *d* to assess the magnitude of group differences. No multiple comparisons correction was done given the *a priori* nature of the hypothesis of lower NAA/Cho as a function of familial proximity to the schizophrenia proband among biological relatives.

3. Results

3.1. Subjects studied

Sociodemographic features of the participants and metabolite ratios from the left hippocampus and ACC are summarized in Table 1. There were no significant age or gender differences between the 3 comparison groups ($F=1.18$ or $\chi^2=0.71$, $p=0.28$). Total subject count in the study was 36 relatives and 25 controls. For the left hippocampus, 2 spectra were excluded in each group for technical reasons, therefore 35 hippocampal spectra from relatives (2nd degree relatives, $N=20$; 1st degree relatives, $N=15$) and 24 from controls were used. Since 17 subjects (12 relatives and 5 controls) had the ACC MRS performed with CSI instead of SVS technique, ACC MRS data for these subjects without SVS data were excluded from this analysis focused on SVS data. Therefore, 24 ACC spectra were available for relatives (2nd degree relatives, $N=12$; 1st degree relatives, $N=12$) and 20 for controls.

3.2. Comparison of relatives with HNV

Significant main effects of group were found on NAA/Cho ratios for both the left hippocampus and ACC (see Table 1 and Fig. 3). Increased schizophrenia risk was associated with stepwise lowering of NAA/Cho in the left hippocampus ($F=6.11$, $p=0.02$) and ACC ($F=4.89$, $p=0.03$) and elevation in Cho/Cr in the left hippocampus ($F=5.55$, $p=0.02$). Mean NAA/Cho and Cho/Cr levels among second-degree relatives of schizophrenia probands were intermediate between metabolite levels of HNV and first-degree relatives of probands. Relationships between schizophrenia risk and left hippocampus Glx/Cho approached but did not achieve statistical significance ($F=3.00$, $p=0.09$); first-degree relatives had lower left hippocampus Glx/Cho. There were no significant associations between schizophrenia risk and the remaining metabolite ratios ($F=2.65$, $p=0.11$). These findings were unchanged when the sample was restricted to relatives without psychiatric diagnoses (i.e. excluding 7 relatives with psychiatric disorders; results available from authors upon request).

The magnitude of metabolite differences between first-degree relatives of schizophrenia probands and HNV was of moderate to large effect sizes (see Table 1; range of Cohen's $d=0.39$ to 1.0 ; median= 0.57). First-degree versus second-degree relative metabolite differences were also moderately large (median $d=0.52$). In contrast, metabolite differences between second-degree relatives and HNV were generally of small to moderate effect sizes (range $d=0.08$ to 0.61 ; median= 0.3). For NAA/Cho ratios, which were significantly associated with schizophrenia risk, reductions in first-degree relatives compared to HNV were of large effect size (Fig. 3a and b; $d=0.83$). Similarly, first-degree relatives had large effect size elevations in Cho/Cr ratios compared to HNV (Fig. 3c; $d=1.0$).

4. Discussion

4.1. Summary of results

The main finding of this study is that susceptibility for schizophrenia is associated with reduced NAA/Cho ratio in the left hippocampus and ACC, and with increased left hippocampus Cho/Cr ratio. Increased familial proximity to the schizophrenia proband among biological relatives correlated with greater magnitude of $^1\text{HMRs}$ metabolite abnormalities. Given that reduced NAA has been consistently linked with schizophrenia probands, the $^1\text{HMRs}$ metabolite abnormalities observed among young, nonpsychotic biological relatives in this study are likely related to shared genetic vulnerability factors. Our findings provide additional support for limbic metabolites as an intermediate phenotype of schizophrenia potentially useful as biomarkers for the quantification of schizophrenia susceptibility.

4.2. Limitations

Before discussing these findings, limitations of this study should be pointed out. The possibility of inclusion of pre-morbid cases is an unavoidable limitation given the cross sectional nature of this study. The lack of $^1\text{HMRs}$ coregistration with segmented MRIs into gray and white matter and CSF prevented determination of absolute metabolite concentrations. Therefore, metabolic ratios were used to account for partial volume effects. Metabolite ratios increase uncertainty since differences may theoretically be due to change in the numerator and/or denominator. However, ratios are particularly useful when the constituent metabolites change in different directions, such as the pattern of increased choline and reduced NAA reported in schizophrenia (Steen et al., 2005). In this scenario, larger changes are expected in the NAA/Cho ratio than in either metabolite alone. Furthermore, metabolite ratios are independent of brain atrophy (Barker et al., 2010). This is especially relevant since biological relatives of schizophrenia probands have smaller frontotemporal brain volumes than HNV, including hippocampal volume deficits (Nelson et al., 1998; Ho and Magnotta, 2010).

4.3. Previous studies

Schizophrenia has been conceptualized as a neurodevelopmental disorder (Murray and Lewis, 1987; Weinberger, 1987; Keshavan, 1999; Fatemi and Folsom, 2009). The disorder typically presents with the first psychotic episode during late adolescence or early adulthood. Anatomic studies in schizophrenia support pathologic changes in limbic structures including the hippocampus and the ACC as well as the dorsolateral prefrontal cortex and superior temporal gyrus (Wright et al., 2000; Honea et al., 2005; Steen et al., 2006; Fatemi and Folsom, 2009). In keeping with neuropathological and imaging studies, NAA deficits in gray and white matter of the frontal lobes and hippocampus are consistently reported in $^1\text{HMRs}$ studies of schizophrenia probands (Steen et al., 2005). Biological relatives of schizophrenia patients are at higher risk to develop schizophrenia than the general population. Brain structural changes have been reported in unaffected relatives of

schizophrenic subjects, who displayed increased left ventricular volumes (Sharma et al., 1998) and reduced volume in the frontotemporal brain and hippocampus (Nelson et al., 1998; Ho and Magnotta, 2010). Recent ¹HMRS studies reported metabolite differences between unaffected relatives of schizophrenia proband patients and HNV. Offspring of schizophrenia probands have reduced medial frontal NAA/Cho (Keshavan et al., 1997) and increased glutamate/glutamine (Tibbo et al., 2004) compared to controls. Reduced metabolic concentrations in relatives compared to HNV were demonstrated in the striatum (Keshavan et al., 2009) and thalamus (Yoo et al., 2009). Moreover, at risk symptomatic subjects demonstrated reduced NAA and increased choline in the left frontal lobe and ACC (Jessen et al., 2006). Results have been heterogeneous mainly because of differences in anatomic regions sampled, ¹HMRS techniques and age of relatives and HNV included.

4.4. Metabolic differences between relatives and HNV

In our study, young unaffected 1st and 2nd degree relatives of schizophrenia probands showed reduced NAA/Cho ratio compared to HNV in limbic brain regions including the bilateral ACC and the left hippocampus. The graded increment in NAA/Cho and Cho/Cr effect sizes corresponding to increased familial proximity to the schizophrenia proband among biological relatives suggests that these ¹HMRS metabolites may be sensitive biomarkers of schizophrenia vulnerability. These findings are thus consistent with Keshavan et al. (1997), in which offsprings of schizophrenia probands showed reduced ACC NAA/Cho ratios compared to HNV. On the other hand, we did not find significant reductions in the left hippocampus NAA/Cr as reported previously (Callicott et al., 1998) or increased ACC Glx ratios (Tibbo et al., 2004). There was no significant change in Glx/Cr in relatives of schizophrenia probands in our study; in contradistinction with two recent studies of healthy relatives of schizophrenia probands that sampled different areas of the prefrontal lobe (Purdon et al., 2008; Lutkenhoff et al., 2010) and the left hippocampus (Lutkenhoff et al., 2010). Differences in MRS methodology including sequence parameters and signal quantitation strategy, anatomical areas explored, and different study populations may explain conflicting results.

4.4.1. NAA—The high brain NAA concentration makes the proton NAA signal a reliable marker in ¹HMRS studies. However, the functional role of NAA in the brain has been less clear. Several hypothesized functions have been proposed, including involvement in neuronal osmotic regulation, N-acetylaspartylglutamate biosynthesis, oligodendrocytic myelin production and neuronal energy metabolism (Moffett et al., 2007). Since NAA is considered a neuronal specific marker in the adult brain (Birken and Oldendorf, 1989), reduced NAA suggests neuronal loss or metabolic impairment (Demougeot et al., 2001). In addition, decreased NAA levels may further reflect compromised viability of remaining neurons or abnormal axonal integrity. In schizophrenia, neuronal loss is not a prominent neuropathological feature (Harrison, 1999). Therefore, the underlying basis for reduced NAA remains uncertain and could reflect reduced neuropil and/or neuronal size. Lower NAA in schizophrenia probands (and by extension their biological relatives) has been thought to be a marker for neurodevelopmental disruption in neuronal proliferation, and/or synaptic connectivity and neuroaxonal myelin maintenance (Keshavan et al., 1997; Jessen et al., 2006). Furthermore, NAA/Cho levels in the left hippocampus are modulated by the SNAP-25 genotype (Scherk et al., 2008). The SNAP-25 protein is part of the neurotransmitter exocytosis machinery and is down-regulated in the post-mortem hippocampus in schizophrenia and bipolar disorder.

4.4.2. Choline—The observed increment in the left hippocampal Cho/Cr together with a lower NAA/Cho and an unchanged NAA/Cr suggests that the lower NAA/Cho may be driven by a higher choline signal in relatives compared to HNV. Increased choline

resonance (consisting mostly of glycerophosphocholine and phosphocholine) reflects accelerated membrane turnover, however, *in vivo* ^1H MRS is insensitive for distinguishing among specific choline-containing phospholipid moieties. Increased choline has been reported in frontal and temporal regions in schizophrenia patients and at-risk subjects (Keshavan et al., 1997; O'Neill et al., 2004; Jessen et al., 2006; Shirayama et al., 2009). ^{31}P MRS studies in schizophrenia probands showed differences in concentration of choline-containing phosphomonoesters and phosphodiesters (Keshavan et al., 2000) which underlie the change in the choline proton signal. By combining the likelihood of both NAA and choline change in a single ratio, such strategy is expected to increase sensitivity to detect subtle metabolite changes. This is consistent with previous reports on NAA/Cho differences between schizophrenia patients and/or their relatives and HNV (Keshavan et al., 1997; Block et al., 2000; Jessen et al., 2006). Furthermore, in clinically-defined at risk groups of symptomatic subjects, NAA/Cho in the ACC was significantly reduced among those who converted to schizophrenia compared to those who did not convert, while there were no significant differences in NAA/Cr (Jessen et al., 2006).

5. Conclusion

The rates of schizophrenia among first-degree biological relatives of schizophrenia probands range between ~50% in monozygotictwins, 15% in offsprings, and ~10% among siblings. Second-degree relatives show lower rates of the disorder (2–5%). In the current study, left hippocampus and ACC NAA/Cho ratios and left hippocampus Cho/Cr ratios in second-degree relatives were intermediate between levels of HNV and first-degree relatives. The magnitudes of ^1H MRS metabolite abnormalities among second-degree relatives compared to HNV were smaller than those in first-degree relatives. Such graded increments in NAA/Cho and Cho/Cr effect sizes corresponding to increased familial proximity to the schizophrenia proband among biological relatives suggest that ^1H MRS variables may be sensitive biomarkers of schizophrenia vulnerability. Further longitudinal research is warranted to assess the value of these metabolite changes as predictors of conversion to schizophrenia.

Acknowledgments

This research was supported in part by NIMH grant MH68380, a NARSAD Independent Investigator Award and the Nellie Ball Research Trust. The authors thank Lindsey Fuhrmeister and Marla Kleingartner for their kind assistance with imaging data processing and data collection.

Role of funding source

This research was supported in part by NIMH grant MH68380, a NARSAD Independent Investigator Award and the Nellie Ball Research Trust. The NIMH had no further role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Author Beng-Choon Ho was supported in part by NIMH grant MH68380, a NARSAD Independent Investigator Award and the Nellie Ball Research Trust.

References

- Abbott C, Bustillo J. What have we learned from proton magnetic resonance spectroscopy about schizophrenia? A critical update. *Current Opinion in Psychiatry*. 2006; 19(2):135–139. [PubMed: 16612192]
- Andreasen NC, Endicott J, Spitzer RL, Winokur G. The family history method using diagnostic criteria. Reliability and validity. *Archives of General Psychiatry*. 1977; 34(10):1229–1235. [PubMed: 911222]
- Andreasen NC, Flaum M, Arndt S. The comprehensive assessment of symptoms and history (CASH). An instrument for assessing diagnosis and psychopathology. *Archives of General Psychiatry*. 1992; 49(8):615–623. [PubMed: 1637251]

- Barker, PB.; Bizzi, A.; De Stefano, N.; Gullapalli, RP.; Lin, DDM. Clinical MR Spectroscopy Techniques and Applications. 1st ed.. Cambridge University Press; New York: 2010.
- Birken DL, Oldendorf WH. N-acetyl-L-aspartic acid: a literature review of a compound prominent in ^1H -NMR spectroscopic studies of brain. *Neuroscience and Biobehavioral Reviews*. 1989; 13(1):23–31. [PubMed: 2671831]
- Block W, Bayer TA, Tepest R, Träber F, Rietschel M, Müller DJ, Schulze TG, Honer WG, Maier W, Schild HH, et al. Decreased frontal lobe ratio of N-acetyl aspartate to choline in familial schizophrenia: a proton magnetic resonance spectroscopy study. *Neuroscience Letters*. 2000; 289(2):147–151. [PubMed: 10904141]
- Braun CM, Boulanger Y, Labelle M, Khiat A, Dumont M, Mailloux C. Brain metabolic differences as a function of hemisphere, writing hand preference, and gender. *Laterality*. 2002; 7(2):97–113. [PubMed: 15513191]
- Callicott JH, Egan MF, Bertolino A, Mattay VS, Langheim FJP, Frank JA, Weinberger DR. Hippocampal N-acetyl aspartate in unaffected siblings of patients with schizophrenia: a possible intermediate neurobiological phenotype. *Biological Psychiatry*. 1998; 44(10):941–950. [PubMed: 9821558]
- Capizzano, AA.; Jorge, RE.; Acion, L.; Magnotta, V. Reproducibility of short TE MRS at 3 Tesla: comparison of hippocampal with cingulate spectra; Proceedings of the XIV Scientific Meeting of the ISMRM; 2006;
- Demougeot C, Garnier P, Mossiat C, Bertrand N, Giroud M, Beley A, Marie C. N-acetylaspartate, a marker of both cellular dysfunction and neuronal loss: its relevance to studies of acute brain injury. *Journal of Neurochemistry*. 2001; 77(2):408–415. [PubMed: 11299303]
- Fatemi SH, Folsom TD. The neurodevelopmental hypothesis of schizophrenia, revisited. *Schizophrenia Bulletin*. 2009; 35(3):528–548. [PubMed: 19223657]
- Haga KK, Khor YP, Farrall A, Wardlaw JM. A systematic review of brain metabolite changes, measured with ^1H magnetic resonance spectroscopy, in healthy aging. *Neurobiology of Aging*. 2009; 30(3):353–363. [PubMed: 17719145]
- Harrison PJ. The neuropathology of schizophrenia: a critical review of the data and their interpretation. *Brain*. 1999; 122(4):593–624. [PubMed: 10219775]
- Ho B, Magnotta V. Hippocampal volume deficits and shape deformities in young biological relatives of schizophrenia probands. *NeuroImage*. 2010; 49(4):3385–3393. [PubMed: 19941961]
- Honea R, Crow TJ, Passingham D, Mackay CE. Regional deficits in brain volume in schizophrenia: a meta-analysis of voxel-based morphometry studies. *American Journal of Psychiatry*. 2005; 162(12):2233–2245. [PubMed: 16330585]
- Jessen F, Scherk H, Traber F, Theyson S, Berning J, Tepest R, Falkai P, Schild HH, Maier W, Wagner M, et al. Proton magnetic resonance spectroscopy in subjects at risk for schizophrenia. *Schizophrenia Research*. 2006; 87(1-3):81–88. [PubMed: 16842971]
- Keshavan MS. Development, disease and degeneration in schizophrenia: a unitary pathophysiological model. *Journal of Psychiatric Research*. 1999; 33(6):513–521. [PubMed: 10628528]
- Keshavan MS, Montrose DM, Pierri JN, Dick EL, Rosenberg D, Talagala L, Sweeney JA. Magnetic resonance imaging and spectroscopy in offspring at risk for schizophrenia: preliminary studies. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*. 1997; 21(8):1285–1295. [PubMed: 9460092]
- Keshavan MS, Stanley JA, Pettegrew JW. Magnetic resonance spectroscopy in schizophrenia: methodological issues and findings—part II. *Biological Psychiatry*. 2000; 48(5):369–380. [PubMed: 10978720]
- Keshavan MS, Dick RM, Diwadkar VA, Montrose DM, Prasad KM, Stanley JA. Striatal metabolic alterations in non-psychotic adolescent offspring at risk for schizophrenia: a ^1H spectroscopy study. *Schizophrenia Research*. 2009; 115(1):88–93. [PubMed: 19748228]
- Lutkenhoff ES, van Erp TG, Thomas MA, Therman S, Manninen M, Huttunen MO, Kaprio J, Lonnqvist J, O'Neill J, Cannon TD. Proton MRS in twin pairs discordant for schizophrenia. *Molecular Psychiatry*. 2010; 15(3):308–318. [PubMed: 18645571]

- Maudsley A, Domenig C, Govind V, Darkazanli A, Studholme C, Arheart K, Bloomer C. Mapping of brain metabolite distributions by volumetric proton MR spectroscopic imaging (MRSI). *Magnetic Resonance in Medicine*. 2009; 61(3):548–559. [PubMed: 19111009]
- Moffett JR, Ross B, Arun P, Madhavarao CN, Namboodiri AMA. N-acetylaspartate in the CNS: from neurodiagnostics to neurobiology. *Progress in Neurobiology*. 2007; 81(2):89–131. [PubMed: 17275978]
- Murray RM, Lewis SW. Is schizophrenia a neurodevelopmental disorder? *British Medical Journal (Clinical Research Ed.)*. 1987; 295(6600):681–682. [PubMed: 3117295]
- Nelson MD, Saykin AJ, Flashman LA, Riordan HJ. Hippocampal volume reduction in schizophrenia as assessed by magnetic resonance imaging: a meta-analytic study. *Archives of General Psychiatry*. 1998; 55(5):433–440. [PubMed: 9596046]
- O'Neill J, Levitt J, Caplan R, Asarnow R, McCracken JT, Toga AW, Alger JR. ¹H MRSI evidence of metabolic abnormalities in childhood-onset schizophrenia. *NeuroImage*. 2004; 21(4):1781–1789. [PubMed: 15050598]
- Provencher SW. Automatic quantitation of localized in vivo ¹H spectra with LCModel. *NMR in Biomedicine*. 2001; 14(4):260–264. [PubMed: 11410943]
- Purdon SE, Valiakalayil A, Hanstock CC, Seres P, Tibbo P. Elevated 3T proton MRS glutamate levels associated with poor continuous performance test (CPT-0X) scores and genetic risk for schizophrenia. *Schizophrenia Research*. 2008; 99(1-3):218–224. [PubMed: 18248960]
- Raininko R, Mattsson P. Metabolite concentrations in supraventricular white matter from teenage to early old age: a short echo time ¹H magnetic resonance spectroscopy (MRS) study. *Acta Radiologica*. 2010; 51(3):309–315. [PubMed: 20170295]
- cherk H, Backens M, Zill P, Schneider-Axmann T, Wobrock T, Usher J, Reith W, Falkai P, Moller HJ, Bondy B, et al. SNAP-25 genotype influences NAA/Cho in left hippocampus. *Journal of Neural Transmission (Vienna, Austria)*. 1996; 2008; 115(11):1513–1518.
- Sharma T, Lancaster E, Lee D, Lewis S, Sigmundsson T, Takei N, Gurling H, Barta P, Pearlson G, Murray R. Brain changes in schizophrenia. Volumetric MRI study of families multiply affected with schizophrenia—the Maudsley Family Study 5. *The British Journal of Psychiatry*. 1998; 173(2):132–138. [PubMed: 9850225]
- Shirayama Y, Obata T, Matsuzawa D, Nonaka H, Kanazawa Y, Yoshitome E, Ikehira H, Hashimoto K, Iyo M. Specific metabolites in the medial prefrontal cortex are associated with the neurocognitive deficits in schizophrenia: a preliminary study. *NeuroImage*. 2009; 49(2010):2783–2790. [PubMed: 19850131]
- Steen RG, Hamer RM, Lieberman JA. Measurement of brain metabolites by ¹H magnetic resonance spectroscopy in patients with schizophrenia: a systematic review and meta-analysis. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*. 2005; 30(11):1949–1962. [PubMed: 16123764]
- Steen RG, Mull C, McClure R, Hamer RM, Lieberman JA. Brain volume in first-episode schizophrenia: systematic review and meta-analysis of magnetic resonance imaging studies. *The British Journal of Psychiatry: The Journal of Mental Science*. 2006; 188:510–518. [PubMed: 16738340]
- Tibbo P, Hanstock C, Valiakalayil A, Allen P. 3-T proton MRS investigation of glutamate and glutamine in adolescents at high genetic risk for schizophrenia. *The American Journal of Psychiatry*. 2004; 161(6):1116–1118. [PubMed: 15169703]
- Weinberger DR. Implications of normal brain development for the pathogenesis of schizophrenia. *Archives of General Psychiatry*. 1987; 44(7):660–669. [PubMed: 3606332]
- Wright IC, Rabe-Hesketh S, Woodruff PW, David AS, Murray RM, Bullmore ET. Meta-analysis of regional brain volumes in schizophrenia. *The American Journal of Psychiatry*. 2000; 157(1):16–25. [PubMed: 10618008]
- Yoo SY, Yeon S, Choi CH, Kang DH, Lee JM, Shin NY, Jung WH, Choi JS, Jang DP, Kwon JS. Proton magnetic resonance spectroscopy in subjects with high genetic risk of schizophrenia: investigation of anterior cingulate, dorsolateral prefrontal cortex and thalamus. *Schizophrenia Research*. 2009; 111(1-3):86–93. [PubMed: 19406622]

Fig. 1.
a: Placement of the VOI for PRES SVS at the left hippocampus. b: Placement of the VOI for PRES SVS at the anterior cingulate cortex.

Fig. 2.
a: LCMoDel output for spectrum from the anterior cingulate cortex. b: LCMoDel output for spectrum from the left hippocampus.

Fig. 3. Metabolite ratios and Cohen's d effect sizes in the left hippocampus and anterior cingulate in 1st and 2nd degree relatives of schizophrenia probands and HNV.

Table 1

Comparison of sociodemographic characteristics and magnetic resonance spectroscopy metabolite ratios between healthy normal volunteers (HNV) without a family history of schizophrenia and first- and second-degree relatives of schizophrenia probands.

	HNV (1)	2nd degree (2)	1st degree (3)	χ^2/F (p)	
N	25	20	16		
Age (years)	20.23 (2.86)	19.51 (3.06)	19.31 (2.34)	1.18 (0.28)	
Males (%)	60.00	55.00	68.75	0.71 (0.70)	
Psychiatric diagnosis (%)	0	4 ^a	3 ^b		
	Mean (SD)	Mean (SD)	Mean (SD)	F (p)	Effect size (Cohen's d)
Left hippocampus^c					
NAA/Cho	5.09 (0.84)	4.76 (0.81)	4.45 (0.62)	6.11 (0.02)	0.40
NAA/Cr	1.49 (0.19)	1.43 (0.21)	1.42 (0.16)	1.21 (0.28)	0.30
Glx/Cho	6.41 (1.16)	6.52 (1.24)	5.54 (1.29)	3.00 (0.09)	0.09
Glx/Cr	1.89 (0.30)	1.96 (0.31)	1.76 (0.30)	0.87 (0.36)	0.23
Cho/Cr	0.29 (0.03)	0.30 (0.03)	0.32 (0.03)	5.55 (0.02)	0.33
Anterior cingulate^d					
NAA/Cho	6.44 (0.88)	5.87 (1.03)	5.70 (0.91)	4.89 (0.03)	0.61
NAA/Cr	1.45 (0.21)	1.37 (0.11)	1.34 (0.16)	2.69 (0.11)	0.44
Glx/Cho	9.44 (1.96)	9.01 (2.15)	10.18 (1.53)	0.69 (0.41)	0.21
Glx/Cr	2.11 (0.41)	2.08 (0.34)	2.33 (0.32)	2.09 (0.16)	0.08
Cho/Cr	0.22 (0.03)	0.24 (0.04)	0.24 (0.02)	1.32 (0.26)	0.29

Note: choline signal is automatically corrected for the number of protons (9) by LCModel, yielding three times lower NAA/Cho ratios as when the correction is not applied since the NAA group has only 3 protons, same as creatine. Bold font indicates statistically significant findings at $p < 0.05$.

^a 2 subjects with major depressive disorder, 1 attention deficit hyperactivity disorder, 1 bipolar II disorder.

^b 1 depressive disorder, not otherwise specified, 2 attention deficit hyperactivity disorder.

^c Sample size: HNV, N = 24; 2nd degree relatives, N = 20; 1st degree relatives, N = 15.

^d Sample size: HNV, N = 20; 2nd degree relatives, N = 12; 1st degree relatives, N = 12.