Preliminary Findings Associate Hippocampal $^1$H-MR Spectroscopic Metabolite Concentrations with Psychotic and Manic Symptoms in Patients with Schizophrenia

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ABSTRACT

BACKGROUND AND PURPOSE: Previous hippocampal proton MR spectroscopic imaging distinguished patients with schizophrenia from controls by elevated Cr levels and significantly more variable NAA and Cho concentrations. This goal of this study was to ascertain whether this metabolic variability is associated with clinical features of the syndrome, possibly reflecting heterogeneous hippocampal pathologies and perhaps variability in its “positive” (psychotic) and “negative” (social and emotional deficits) symptoms.

MATERIALS AND METHODS: In a sample of 15 patients with schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, we examined the association of NAA and Cho levels with research diagnostic interviews and clinical symptom ratings of the patients. Metabolite concentrations were previously obtained with 3D proton MR spectroscopic imaging at 3T, a technique that facilitates complete coverage of this small, irregularly shaped, bilateral, temporal lobe structure.

RESULTS: The patient cohort comprised 8 men and 7 women (mean age, 39.1 [SD, 10.8] years, with a mean disease duration of 17.2 [SD, 10.8] years. Despite the relatively modest cohort size, we found the following: 1) Elevated Cho levels predict the positive (psychotic, $r = 0.590, P = .021$) and manic ($r = 0.686, P = .005$) symptom severity; and 2) lower NAA levels trend toward negative symptoms ($r = 0.484, P = .08$). No clinical symptoms were associated with Cr level or hippocampal volume (all, $P \geq .055$).

CONCLUSIONS: These preliminary findings suggest that NAA and Cho variations reflect different pathophysiologic processes, consistent with microgliosis/astrogliosis and/or lower vitality (reduced NAA) and demyelination (elevated Cho). In particular, the active state-related symptoms, including psychosis and mania, were associated with demyelination. Consequently, their deviations from the means of healthy controls may be a marker that may benefit precision medicine in selection and monitoring of schizophrenia treatment.

ABBREVIATIONS: AP = anterior-posterior; $^1$H-MRSI = proton MR spectroscopic imaging; IS = inferior-superior; LR = left-right

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cizophrenia and related psychoses are debilitating disorders affecting $\geq 1\%$ of the population. Its clinical features include psychosis (hallucinations, delusions, disorganization) and decreased emotional expression and avolition (negative symptoms), along with declining function and frequent mood symptoms. While commonly approached and treated as 1 disorder, schizophrenia is a heterogeneous syndrome with mounting evidence that different genetic susceptibilities and exposures may produce different initiating pathophysiology.

Hippocampal disruption may be a central pathology for psychosis because many of its measures differ between patients with schizophrenia and healthy controls, eg, reduced volume, increased resting blood flow, impaired task-related activation, decreased neurogenesis, and reduced connectivity with other regions. Because the disease entails cognitive and attention deficits, effort-independent methods, eg, 3D proton MR spectroscopic imaging ($^1$H-MRSI) of the hippocampal formation, are well-suited to both elucidating the etiology of schizophrenia and monitoring its treatments. $^1$H-MRSI yields metabolic markers of several cellular processes, most notably NAA for neuronal integrity, Cr (phosphocreatine and creatine) for energy metabolism, Cho (choline, phosphocholine, and glycerophosphocholine) for membrane turnover and astroglia proliferation, and mIns for inflammation and gliosis.
Most hippocampus 1H-MR spectroscopy literature reports on schizophrenia consider group-level differences in metabolite concentrations between patients and matched healthy controls. For example, a recent whole-hippocampus 1H-MRSI study of patients with schizophrenia found only significant, 19%, elevated mean Cr concentrations and 10% lower structure volume compared with healthy controls. Although the mean NAA and Cho levels in that study did not differ between the 2 cohorts, their within-group variability was significantly greater in the schizophrenia group than in the controls. While this greater variance may have reduced statistical power to detect group differences, it may come as no surprise, considering the etiologic and clinical heterogeneity of the disease and myriad treatment paradigms and durations.

Because this heterogeneity impedes therapeutic success, there is an ongoing research to define biologic subtypes with clinical significance. Because 1H-MR spectroscopy is often used to identify disease markers leading to personalized treatments, we set out to investigate whether this variability of Cho and NAA levels in patients with schizophrenia could reflect clinical symptoms, even absent group differences from the controls. This report presents preliminary findings and analysis of NAA, Cr, Cho, and mIns variability with respect to the clinical symptoms of patients with schizophrenia to test the hypothesis that their variations reflect the patient’s clinical presentation.

### MATERIALS AND METHODS

#### Participants

Subjects were recruited from a pool of 19 patients with established schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, who participated in a previous 1H-MRSI and MR imaging study. Fifteen (9 men, 6 women, mean age 41.60 [SD, 10.8] years; mean disease duration 18 [SD, 10.02] years) consented to enroll (see Table 1 for demographic information). All were on stable medication regimens for at least a month before their recruitment. They were assessed with the Positive and Negative Syndrome Scale, to generate ratings for positive (psychotic), negative (social and emotional deficits), and general psychopathology statuses. Depression and anxiety were assessed with the Hamilton Depression Rating Scale and the Hamilton Anxiety Rating Scale; the Young Mania Rating Scale was used to assess manic symptoms, all compiled in Table 2. All subjects provided New York University School of Medicine Institutional Review Board–approved written informed consent.

#### MR Imaging Acquisition and Postprocessing

MR imaging and 1H-MRSI were performed on a 3T whole-body MR imaging scanner (Trio; Siemens) with an after-market, circularly-polarized transmit-receive head coil (TEM-3000 MRInstruments Inc. Minneapolis MN). For tissue segmentation and 1H-MRSI VOI guidance, T1-weighted, 3D MPPAGE images were obtained from each subject: TE/TE/TR = 2.6/800/1360 ms, matrix = 256 × 256, FOV = 256 × 256 mm², 1-mm-thick slices = 160, reformatted into sagittal and coronal slices at 1-mm³ isotropic resolution, tilted along the hippocampal axis, as shown in Fig 1A–C.

A 6-cm anterior–posterior (AP) × 9-cm left–right (LR) × 2-cm inferior–superior (IS) VOI was then image-guided over the bilateral hippocampi (Fig 1A–C), angled along its long axis, and excited into 4 Hadamard-encoded axial slices, with a point-resolved spectroscopy sequence (TE/TR = 35/1400 ms). It yielded 6 × 9 × 4 (AP × LR × IS) = 216 voxels in the VOI, 1.0 × 1.0 × 0.5 cm³ each, as shown in Fig 1A–C. The 3D 1H-MRSI data were reconstructed off-line using in-house software, as shown in Fig 1D. The relative NAA, Cr, Cho, and mIns levels of each voxel were obtained with the spectral modeling package of Soher et al as shown in Fig 1E and scaled into absolute millimolar concentrations with phantom replacement, according to Meyer et al.

Bilateral (left + right) hippocampal masks were traced by a trained neuroradiologist on the original sagittal MPRAge images. The tracing was based on the well-known, extensively validated Harmonized Protocol, developed by the European Alzheimer’s Disease Consortium. It prescribes guidelines for labeling the entire hippocampal formation from native-space high-resolution T1-weighted MR imaging. To edit and inspect the 3D hippocampal masks in axial, sagittal, and coronal views, we used the FireVoxel software.

### Table 1: Demographics, metabolic, and symptomatic characteristics of the 15 patients, arranged in ascending age

| No. | Age a | Sex | Disease Duration a | NAA b | Cr b | Cho b | Volume c |
|-----|-------|-----|-------------------|-------|------|-------|----------|
| 1   | 23    | M   | 3                 | 10.57 | 10.91 | 2.76  | 8.56     |
| 2   | 23    | M   | 4                 | 8.42  | 10.8  | 2.5   | 7.99     |
| 3   | 29    | F   | 8                 | 10.6  | 10.51 | 2.37  | 8.42     |
| 4   | 34    | M   | 5                 | 11.31 | 10.77 | 2.03  | 7.71     |
| 5   | 41    | M   | 22                | 8.91  | 7.2   | 2.35  | 5.71     |
| 6   | 42    | M   | 18                | 4.95  | 4.44  | 1.84  | 8.84     |
| 7   | 42    | F   | 20                | 9.62  | 10.6  | 3.31  | 7.16     |
| 8   | 42    | F   | 23                | 9.81  | 7.72  | 1.46  | 7.18     |
| 9   | 44    | M   | 26                | 9.04  | 4.46  | 3.85  | 8.78     |
| 10  | 48    | M   | 23                | 9.36  | 9.44  | 2.9   | 7.41     |
| 11  | 49    | F   | 31                | -     | 12.01 | 1.75  | 6.83     |
| 12  | 51    | F   | 10                | 7.74  | 10.07 | 3.28  | 6.65     |
| 13  | 52    | M   | 32                | 8.43  | 8.68  | 1.88  | 7.11     |
| 14  | 52    | M   | 30                | 15.69 | 7.15  | 4.56  | 7.16     |
| 15  | 52    | F   | 15                | 8.38  | 8.7   | 2.15  | 5.9      |
| Mean ± SD | 41.60 [10.08] | 18 [10.02] | 9.49 [2.35] | 8.90 [2.31] | 2.60 [0.86] | 7.43 [0.97] |

Note: — indicates missing value.
a Years.
b Millimolar.
c Bilateral hippocampus volume, cubic centimeters.
package (https://firevoxel.org). FireVoxel then resampled the left +
right hippocampal masks along the long axis of each subject’s hip-
campus at the same inclination (ie, parallel and naturally coregis-
tered) to the 1H-MRSI VOI, as shown in Fig 1. The CSF and white
matter partial volume–corrected 1H-MRSI metabolite concentra-
tions within each subject’s hippocampus mask, as well as the volume
of the masks themselves, were then estimated using the Matlab soft-
ware package (MathWorks), described by Tal et al.25

Statistical Analyses

SPSS (Version 21.0; IBM) was used for analyses. The data were
normally distributed (all Kolmogorov–Smirnov test P values were
P > .055). No clinical symptoms were associated with Cr
values (P = .09); thus, the association between clinical symptom ratings and the
Cho, NAA, Cr levels and hippocampal volumes were examined using Pearson correlations. All tests were 2-tailed, and the α for
significance was set at P < .05.

RESULTS

Individual scores and sample means ± SDs for the hippocampal
metabolite concentrations and volumes are compiled in Table 1,
and the psychiatric symptoms are presented in Table 2. There was
a positive association between Cho levels and the Positive and
Negative Syndrome Scale–positive symptoms (r = 0.59, P = .021)
and manic symptoms (r = 0.69, P = .005), as shown in Fig 2. There
was a trend association of the NAA with negative symptoms
(r = 0.48, P = .08). No clinical symptoms were associated with Cr
levels or the hippocampal volume (all, P values ≥ .055).

DISCUSSION

Most of the 1H-MR spectroscopy schizophrenia studies published
to date focus on the search for group-concentration differences
from matched healthy controls.14 The usual rationale is the search for a singular underlying disease pathology, rather than a
clinical phenotype within the syndrome. Moreover, the variability
in metabolite concentrations that could be used to explore differ-
ent subtypes of the disease with different molecular pathologies
may, instead, reduce the power to find group differences. Thus,
approaching schizophrenia as a singular condition (though
acknowledged to be a heterogeneous syndrome) may lead to the
findings of “no intercohort difference.” This may explain the pau-

city of Cho, mIns, and Cr patient-versus-control intercohort dif-
ference reports in the literature and perhaps, consequently, the
even greater rarity of these concentration relationships to the
patient’s clinical symptoms.

These preliminary findings support the hypothesis that some of
the variability in the patient’s clinical symptoms is related to the hip-
campus metabolite concentrations and the different molecular
pathologies they indicate. This is especially germane because bio-
markers that follow clinical symptoms would be particularly helpful
in early-psychosis groups, including first-episode, prodromal, and
clinical high-risk subjects. For example, during early-psychosis pre-
sentation, psychiatric history is often limited, but successful long-
term outcomes rely on accurate diagnosis and quick intervention.26

Identifying molecular pathologies in the hippocampus is particu-
larly poignant in this population. Indeed, studies of subjects at high
risk for psychosis have identified changes in hippocampal volume
and glutamate and glutamine concentrations.27,28

This study suggests that 2 different hippocampal inflammatory
pathologies may occur in different cases of schizophrenia, identified
by elevated Cho, a marker for demyelination, and reduced NAA,
an indicator of neuronal integrity. In support of the proposition
that these processes are independent, the cases with the highest Cho
levels also had mild NAA elevation. Elevated Cho was specifically
related to state-related active symptoms of psychosis and mania.
Reduced hippocampal NAA is consistent with astrogliosis and/
or microgliosis, shown in a murine model of HIV-1 encephalitis.29
and consistent with the elevated Cr levels, which correlated with mIns levels for the entire group of cases versus controls. The relationship of Cho with the cases of more severe manic and psychotic symptoms suggests that the pathologic processes associated with their active positive symptoms may entail myelination disruption.

Note that the inhibitory parvalbumin-positive GABAergic hippocampal interneurons are myelinated in an experience-dependent manner. With diminished hippocampal input, the number and function of the inhibitory GABAergic neurons would be reduced, shifting the balance to the more numerous N-methyl-D-aspartate receptor glutamatergic activity, thereby activating pathways that can deregulate dopamine homeostasis and produce psychosis. Such a model would support our finding of Cho predicting psychotic and manic symptoms. Furthermore, the fact that Cho, a marker of membrane de- and remyelination, is linked to psychosis severity supports a mechanism of dysmyelination in schizophrenia that is also supported by a postmortem study showing that 35 of 89 cases examined expressed abnormal myelination in the prefrontal lobes.

This study focused on the variability of metabolite concentrations as a probe of the underpinnings of hippocampal pathology in psychosis. The presence of separate hippocampus-related pathologies may help reconcile the diverse findings reported for schizophrenia better than single models that include effects of age or progression of a single pathophysicsology. These findings are also consistent with a model of increased hippocampal hyperactivation or perfusion and pathology that reduces hippocampal volumes.

This study is also subject to some limitations. First, it is a small (n = 15) sample comprising patients of varying disease durations. While all were on clinically determined medication doses, unchanged for at least a month, their treatments varied in both type and duration. Second, the quantitative metabolic results are subject to inherent limitations of 1H-MR spectroscopy: relatively low spatial resolution compared with the hippocampus internal structure, precluding, for example, differentiation of its subfields. Yet, even despite these limitations, our results suggest that individual 1H-MRSI metrics may be used to help characterize the clinical presentation of patients with schizophrenia with hippocampal metabolic deviations compared with healthy controls.

CONCLUSIONS
A combination of advanced multivoxel 3D 1H-MRSI acquisition facilitating total spatial coverage of this bilateral structure and postprocessing methodology that corrects for partial volume effects allowed us to detect and distinguish separate hippocampal pathologies consistent with glial activation and demyelination that predict different psychiatric symptoms in patients with schizophrenia. These preliminary findings, if replicated in a larger study, also suggest that a patient’s specific 1H-MRSI metrics may
be used to select and monitor individually tailored treatments for psychosis and mania; for example, they may benefit from anti-inflammatory treatments.

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