Myelin and Axon Abnormalities in Schizophrenia Measured Using Magnetic Resonance Imaging Techniques

Supplemental Information

Supplemental Methods & Materials

Inclusion/Exclusion Criteria, Screening, and Study Procedures

Participants were men and women between the ages of 18 and 52; the control and schizophrenia (SZ) groups were matched for age and sex. Participants older than 52 were excluded from the study because of the higher burden of white matter (WM) abnormalities starting in the sixth decade (1). All participants reported being right-handed. Participants were excluded if they had significant medical or neurological illness, contraindication to magnetic resonance (MR) scan (including claustrophobia), or pregnancy (screened with a urine test on scan day; females of child-bearing age were using an effective contraceptive method). Control participants were screened using the Structured Clinical Interview for the DSM-IV (SCID-IV) and had no personal history of psychiatric illness including substance abuse or dependence, and no history of the same in first degree relatives. SZ participants fulfilled criteria for SZ or schizoaffective disorder (SZA) according to the DSM-IV, assessed using the SCID-IV. SZA patients (n = 10) were included in this study if they were chronically psychotic and not in a current mood episode. Patients who met criteria for any substance abuse in the past 3 months or a lifetime diagnosis of substance dependence were excluded. Subjects who smoked tobacco were not excluded from the study, but smoking behavior was assessed using the Fagerstrom questionnaire. All but one patient in the SZ group was taking antipsychotic medications, and some were taking additional medications (such as benzodiazepines, lithium, or anticonvulsants).

Chlorpromazine equivalents were calculated for antipsychotic medication dosages for all patients (2).

All participants correctly completed a Consent Survey that asks 10 simple questions about the study, such as "What illness is being studied?" The study visit consisted of consent procedures; a standard clinical evaluation using the SCID-IV; urine toxicology screen; urine pregnancy test, if necessary; 70 minute proton magnetic resonance spectroscopy scan at 4T; 15 minute diagnostic MR imaging scan at 3T, if one had not been obtained within one year (reviewed by radiologist and participants with significant brain abnormalities excluded). The following standardized scales were administered to SZ patients: Positive and Negative Syndrome Scale; Scale for Assessment of Positive Symptoms; Young Mania Rating Scale; Montgomery-Asberg Depression Rating Scale; Multnomah Community Ability Scale; North American Adult Reading Test (a putative measure of premorbid IQ); and Fagerstrom Test for Nicotine Dependence. Body mass index and educational level were also collected for all subjects and lifetime number of suicide attempts and hospitalizations were assessed for patients.

Anatomic Imaging and Voxel Placement

The diagnostic scan was obtained in a Siemens 3 Tesla Trio scanner (Erlangen, Germany); details as in previous publications (3). All magnetization transfer ratio (MTR) and diffusion tensor spectroscopy (DTS) acquisitions were conducted on a 4 Tesla full body MR scanner (Varian/UnityInova, Varian Inc., Palo Alto, California), using a 16-rung, single-tuned, volumetric birdcage coil (Robarts Research Institute, London, Canada). First, a rapid 2D gradient-recalled echo image (12 s) was used to acquire images in three dimensions for rapid determination of subject position and repositioning if necessary. Manual global shimming of

unsuppressed water signal yielded a global water linewidth of ≤ 25 Hz. High-contrast T1-weighted sagittal images (TE/TR = 6.2/11.4 ms, field-of-view (FOV) = 24 x 24 x 8 cm, readout-duration = 4 ms, receive bandwidth = ±32 kHz, data matrix size = 128 x 256 x 16, in-plane resolution = 0.94 x 1.88 mm, slice thickness = 5 mm, readout points = 512, flip angle = 11°) were acquired to serve as an anatomical guide. T1-weighted axial images of the slab TE/TR = 6.2/11.4 ms, FOV = 24 x 24 x 8 cm, readout-duration = 4 ms, receive bandwidth = ±32 kHz, data matrix size = 256 x 256 x 32, in-plane resolution = 0.94 x 0.94 mm, slice thickness = 2.5 mm, readout points = 512, flip-angle = 11°) were then acquired mid-sagittally, allowing for clear differentiation between gray and white matter. In total, imaging time including shimming was approximately 15 minutes. A 1 x 3 x 3 cm^3 single WM voxel was then placed on prefrontal cortex (PFC) of the right hemisphere for MTR and DTS studies. We focused on the WM underlying the PFC because diffusion tensor imaging (DTI) and functional connectivity MRI abnormalities are commonly observed in this brain region (4), and because it affords placement of a large enough voxel on a relatively homogeneous region relevant to the pathophysiology of SZ. Our current protocol can collect good quality data from only one voxel, so we made the decision to collect only right PFC data. DTI abnormalities do not have hemispheric predilection in SZ (4) and we wanted to avoid any DTS signal changes secondary to language-related specialization in the left hemisphere (5). This voxel was placed in the corona radiata, centered at the level of the genu of the corpus callosum but lateral to it (i.e. does not include any callosal fibers). The mediolateral extent was 1 cm, while anteroposterior and dorsoventral extents were 3 cm. The voxel was consistently positioned in pure WM. Its position was anchored by adjacent gray matter in anterior and lateral directions, ensuring that it was placed in comparable location

across scans. We also carried out shimming within the voxel where data were acquired with water resonance linewidths of ≤15 Hz and an average of 12 Hz.

Test-Retest Study and Face Validity of DTS Findings

We carried out a test-retest study to determine within-subject reliability of our measures. Ten healthy subjects (8 male, 2 female; ages 21-47) with no medical/neurological/ psychiatric/substance use problems, and not taking medications, were scanned twice. Scans were conducted within 4 months of one another for each subject. All scans and data analysis were identical to methods described in the manuscript. The within-subject coefficients of variation (CV = standard deviation/mean of 2 scans) were 7.0% for MTR, 3.4% for *N*-acetylaspartate (NAA) apparent diffusion coefficient (ADC), and 1.5% for water ADC. These numbers indicate excellent agreement between measures from the two scans, and suggest that voxel placement and other sources of scan-to-scan variance did not significantly impact our findings.

DTS SNR Measurements

DTS measurements are affected by spectral quality, which is determined by multiple factors including metabolite concentration, T2 transverse relaxation time, as well as participant movement. To address these issues we examined signal-to-noise ratio (SNR) as a measure of spectral quality. Mean NAA SNR was 32.3 for controls and 27.8 for the schizophrenia group. Following application of diffusion gradients, mean NAA SNR was reduced to 21.5 for controls and 15.0 for the schizophrenia group. The lowest NAA SNR for any one subject with or without diffusion gradients applied was 8. In addition, there was no significant correlation between NAA

SNR (with or without diffusion gradients) and NAA ADC (not shown). These results suggest that our study did not suffer from NAA signal sensitivity problems.

Measurement errors due to subject movement were partially reduced by our interleave data acquisition strategy. Nonetheless, we also performed linewidth measurements to check for this artifact. NAA linewidths in the absence and presence of diffusion gradients were 13.1 ± 3.7 and 12.4 ± 3.6 Hz for healthy controls and 16.0 ± 4.6 and 14.9 ± 3.7 Hz for schizophrenia patients, respectively (p = 0.71 and 0.46 for the healthy controls and patients, respectively).

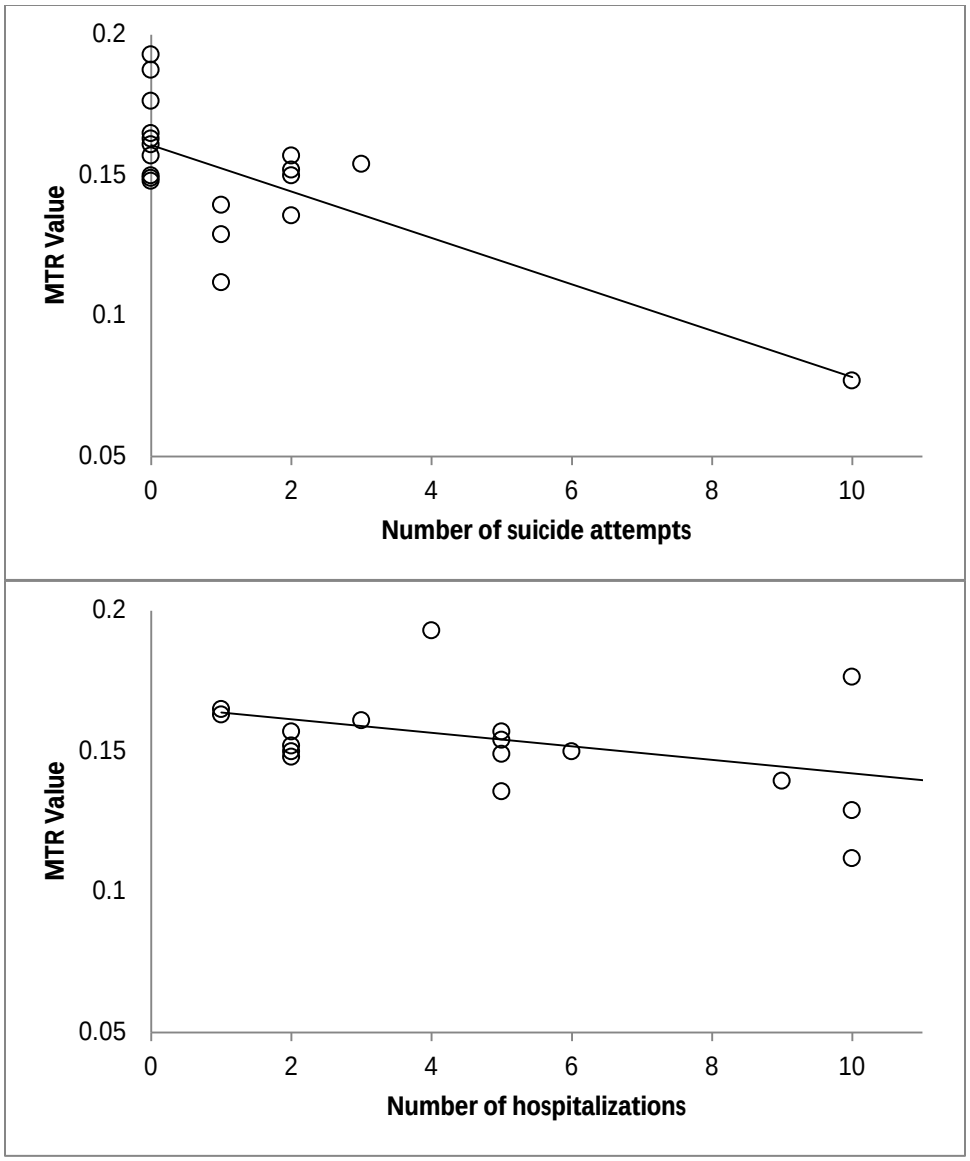

Figure S1. Number of lifetime suicide attempts and number of lifetime hospitalizations among patients. MTR, magnetization transfer ratio.

Supplemental References

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