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Prefrontal neuronal integrity predicts symptoms and cognition in schizophrenia and is sensitive to genetic heterogeneity

Dolores Malaspina [a,*], Thorsten M. Kranz [b], Adriana Heguy [c], Sheila Harroch [d], Robert Mazgaj [a], Karen Rothman [a], Adam Berns [a], Sumya Hasan [a], Daniel Antonius [e], Raymond Goetz [f,j], Mariana Lazar [g,k], Moses V. Chao [b,h,i], Oded Gonen [g,k]

[a] Institute for Social and Psychiatric Initiatives (InSPIRES), Department of Psychiatry, New York University School of Medicine, NY, NY, USA
[b] Skirball Institute of Biomolecular Medicine, Department of Cell Biology, New York University, NY, NY, USA
[c] Genome Technology Center, New York University School of Medicine, NY, NY, USA
[d] Department of Psychiatry, New York University School of Medicine, NY, NY, USA
[e] University at Buffalo, Department of Psychiatry, Buffalo, NY, USA
[f] New York State Psychiatric Institute, Division of Clinical Phenomenology, 1051 Riverside Drive, NY, NY, USA
[g] Center for Advanced Imaging Innovation and Research (CAI2R), Department of Radiology, New York University School of Medicine, NY, NY, USA
[h] Department of Physiology and Neuroscience, Center for Neural Science, New York University, NY, NY, USA
[i] Department of Psychiatry, Center for Neural Science, New York University, NY, NY, USA
[j] Columbia University, Department of Psychiatry, NY, NY, USA
[k] Bernard and Irene Schwartz Center for Biomedical Imaging, Department of Radiology, New York University School of Medicine, NY, NY, USA

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ABSTRACT

Schizophrenia is a genetically complex syndrome with substantial inter-subject variability in multiple domains. Person-specific measures to resolve its heterogeneity could focus on the variability in prefrontal integrity, which this study indexed as relative rostralization within the anterior cingulate cortex (ACC). Twenty-two schizophrenia cases and 11 controls underwent rigorous diagnostic procedures, symptom assessments (PANSS, Deficit Syndrome Scale) and intelligence testing. All underwent multivoxel MRSI at 3 T to measure concentrations of the neuronal-specific biomarker N-acetylaspartate (NAA) in all of the voxels of the ACC. The concentrations of NAA were separately calculated and then compared across the rostral and caudal subregions to generate a rostralization ratio, which was examined with respect to the study measures and to which cases carried a missense coding polymorphism in *PTPRG*, *SCL39A13*, *TGM5*, *NTRK1* or *ARMS/KIDINS220*. Rostralization significantly differed between cases and controls ($\chi^2 = 18.40$, $p < .0001$). In cases, it predicted verbal intelligence ($r = .469$, $p = .043$) and trait negative symptoms (diminished emotional range ($r = -.624$, $p = .010$); curbed interests, $r = -.558$, $p = .025$). Rostralization was similar to controls for missense coding variants in *TGM5* and was significantly greater than controls for the *PTPRG* variant carrier. This is the first study examining the utility of MRS metrics in describing pathological features at both group and person-specific levels. Rostralization predicted core illness features and differed based on which signaling genes were disrupted. While future studies in larger populations are needed, ACC rostralization appears to be a promising measure to reduce the heterogeneity of schizophrenia for genetic research and selecting cases for treatment studies.

1. Introduction

Schizophrenia related psychoses (SRP) are costly and genetically complex brain disorders. Genome wide association studies identify more than a hundred susceptibility loci, but many of these associations are poor and are also associated with bipolar disorder (Frans et al., 2008), autism (Reichenberg et al., 2006) and other conditions (Doherty and Owen, 2014). SRP also shows substantial variability in symptoms, cognition and neuroimaging measures. For person-specific interventions, the field needs simple and effort-independent imaging biomarkers for SRP that are sensitive to established pathophysiological processes. Measures of prefrontal integrity and function have long been sought that would be sensitive to core negative symptoms and cognitive deficits (Egan and Weinberger, 1997). If SRP is a collection of heterogeneous disorders, each strongly influenced by groups of particular susceptibility genes, then a valuable measure would furthermore distinguish gene effects that work through different signaling pathways.

One within-subject and effort independent measure of rostralization is derived as the ratio of N-acetyl-L-aspartate (NAA) in the rostral versus caudal subregion of the anterior cingulate cortex (ACC). NAA is a

* Corresponding author at: Department of Psychiatry, 1 Park Avenue, 8th Floor, Rm 222, New York, NY 10016, USA.
E-mail address: dolores.malaspina@nyumc.org (D. Malaspina).

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neuronal-specific metabolite whose concentration can be measured by Magnetic Resonance Spectroscopic Imaging (MRSI) and which indicates neuronal health and integrity. In a previous study (Hardy et al., 2011), we found that the ratio of NAA in the rostral versus caudal subregion of the ACC was significantly lower in cases than controls, consistent with a neuronal deficit in the rostral subregion in schizophrenia. Notably, the cases showed a significantly greater variability in rostralization than the control group, consistent with the etiological heterogeneity of schizophrenia. Some cases had deficient rostralization, driving the significant difference between the cases and controls, but other cases were either similar to controls and or had greater rostralization than controls. Based on the substantial variability of the biomarker within the cases, the current study re-examined the data with respect to symptoms and cognition and also probed its potential to discriminate among carriers of rare variants in five different major signaling genes. Three were chosen for study in this sample because they harbored de novo missense rare polymorphisms in sporadic schizophrenia cases compared to healthy parents from an Israel sample (*PTPRG*, *SLC39A13*, *TGM5*) (Kranz et al., 2015b) and two selected genes were chosen for their involvement in neurotrophin signaling (*NTRK1*, *ARMS/KIDINS220*) (Kranz et al., 2015a).

2. Materials and methods

2.1. Participants

Outpatient cases with a diagnosis of schizophrenia or schizoaffective disorder maintained on steady medication regimens for at least one month were recruited from treatment settings at Bellevue Hospital Center and healthy comparison subjects were recruited from medical center advertisements. All subjects provided written informed consent. Exclusion criteria included presence of internal metal devices, pregnancy, current substance abuse, use of steroidal contraceptives or allergy medications, and history of epilepsy or head injury requiring medical treatment. The study was approved by the Institutional Review Board as a component of an NIMH Challenge Grant to examine genetics and phenotypic variability among schizophrenia cases.

2.2. Assessments

Diagnosis was based on the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al., 1994) and chart reviews. Cross-sectional symptoms were assessed using the 5-factor model of the Positive and Negative Syndrome Scale (PANSS) as Negative, Positive, Dysphoric Mood, Activation (hostility) and Autistic Preoccupation (Kay et al., 1987; White et al., 1997) and enduring (trait) negative symptoms were rated based on the Schedule for the Deficit Syndrome (Kirkpatrick et al., 1989). Wechsler Adult Intelligence Scale (WAIS-III) (Wechsler, 1999) testing yielded Verbal, Performance and full scale Intelligence Quotients (IQs) as well indices for working memory, perceptual organization, processing speed and verbal comprehension. Research assessments were performed by trained raters at the master's level and above who underwent ongoing evaluations, with inter-rater reliability = 0.95 for DSM-IV diagnoses.

2.3. MR data acquisition

For the main imaging outcome, Single photon MRSI scans separately quantified each subject's absolute N-acetyl-L-aspartate (NAA) and other metabolites' concentrations in the entire rostral and caudal ACC subregions, as shown in Fig. 1, and the relative rostral to caudal concentration ratios were calculated. Briefly, experiments were performed with a 3-T whole-body MR imager (Trio; Siemens, Erlangen Germany) and a transmit-receive head-coil (TEM3000; MR Instruments, Minneapolis, Minn) (Hardy et al., 2011). For anatomic reference and guidance of the spectroscopic volume of interest (VOI), a 3D T_1-weighted magnetization-prepared rapid gradient-echo (MP-RAGE) MRI was acquired: repetition time (TR)/echo time (TE)/inversion time, 1360/2.6/800 ms; 256 × 256 × 160 matrix, and 256 × 256 × 160 mm^3 field of view. The images were reformatted into axial, sagittal and coronal orientations at 1 mm^3 isotropic resolution.

A chemical shift imaging-based procedure subsequently automatically adjusted the first- and second-order magnetic field shims to a whole-head water linewidths of 27 ± 3 Hz in 3–5 min (Tal et al., 2012). An 8 cm anterior–posterior × 5 cm left–right × 3 cm inferior–superior (=120-cm^3) parallelepiped ^{1}H-MRSI VOI was then image-guided over the ACC, as shown in Fig. 1a, b and excited using point-resolved spectroscopy (PRESS): TR/TE = 1800/35 ms. The VOI was partitioned into 8 × 5 × 6 voxels, a nominal 0.5 cm^3 each, as shown in Fig. 1b. The MR signal was acquired for 256 ms at ±1 kHz bandwidth. At two averages, the ^{1}H MRSI took 30 min and the entire protocol lasted less than 1 h.

The data was post processed as described by Hardy et al. (2011). The caudal and rostral ACC were outlined and owing to their irregular shape, manually traced on the axial MRI of each subject, as presented in Fig. 1a, b with software then adding the cerebrospinal fluid-partial-volume-corrected (Soher et al., 1998), phased and aligned spectra from all voxels that fell completely or partially within the circumscribed region, as shown in Figs. 1c, d. In addition to NAA, aspartate, glutamate, glutamine, choline, creatine (Cr), myo-inositol, and taurine model functions were used to fit the data. NAA and Cr relative concentrations were determined for the rostral and caudal ACC using the SiTTools-Fitt software package of Soher et al. (1998), as shown in Fig. 1d. While some studies separate NAA from N-acetylaspartylglutamate (NAAG), which modulates glutamate release, we left these signals combined. It was possible to discern NAA from NAAG (5 Hz apart) in the voxels with the best shim, which were in the dorsal ACC, but the two were unresolved in any of the rostral ACC voxels. As it is only about 0.15 of the NAA amplitude, the SNR would be quite low (1–1.5 mM versus 9 mM for the NAA). Since the fidelity of the quantification could be inexact, we treated the NAAG and NAA as a single resonance.

In all subjects the caudal ACC showed better homogeneity than the rostral subregion. This was due to the proximity of the rostral subregion to the air-filled sinuses, which distort the local magnetic field (air-tissue susceptibility broadening). While this effect makes the spectra look "broader" in the rostral ACC and less "sharp" than in in the caudal subregion, the spectral fitting routine adjusted for line-widths and the quality of metabolic quantification is not adversely affected. Of note, the data was not normalized to a common space (e.g. MNI) and subject to automatic analyses because SPM was only used to segment white matter, gray matter, and cerebrospinal fluid to correct for the partial volume of CSF in each voxel, which is metabolite free. As MRS is of much lower resolution, the matrix size and the alignment process would have distorted the metabolic information. Instead the spectroscopy and anatomical data were aligned in each subject's space and spectra and the associated metrics (i.e., metabolites' concentrations) were obtained in this space.

2.4. Targeted exome capture

This study examined the rostralization ratios of cases with missense coding rare variants (MAF < .01) in any of five genes compared to a group of cases with common sequences in all of these genes. One case each harbored rare variants and novel missense variants in transglutaminase 5 (*TGM5*), zinc transporter ZIP13 (*SLC39A13*), receptor-type tyrosine-protein phosphatase gamma (*PTPRG*), ankyrin rich membrane spanning protein (*ARMS/KIDINS220*) and tyrosine receptor kinase A (*NTRK1*) (Kranz et al., 2015a; Kranz et al., 2015b).

2.5. Data analysis

Data were entered and verified using the SIR Database Management Software (SIR 2002, SIR Pty Ltd) and analyzed with IBM SPSS (Statistics 22). Descriptive statistics and distributions of all continuous and categorical measures were examined to identify features impacting

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