Archival Report

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Lower Posterior Cingulate Cortex Glutathione Levels in Obsessive-Compulsive Disorder

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ABSTRACT

BACKGROUND: Several lines of evidence support the hypothesis that lower cerebral levels of glutathione (GSH), associated with increased oxidative stress, may contribute to obsessive-compulsive disorder (OCD). However, no studies to date have investigated brain GSH levels in individuals with OCD.

METHODS: Twenty-nine individuals with OCD and 25 age-, sex-, and race-matched comparison individuals without OCD underwent single-voxel, two-dimensional J-resolved proton magnetic resonance spectroscopy (MRS) to examine GSH levels in the posterior cingulate cortex (PCC). MRS data were analyzed using LCModel and a simulated basis set. Group metabolite differences referenced to total creatine (Cr), as well as relationships between metabolite ratios and symptom severity as measured by the Yale-Brown Obsessive-Compulsive Scale, were analyzed using linear regression with adjustment for age, sex, and race.

RESULTS: One OCD participant failed to produce usable PCC MRS data. We found significantly lower PCC GSH/Cr in OCD participants compared with non-OCD participants ($\beta = -.027$; 95% confidence interval: $-.049$ to -5.9×10^{-3}; $p = .014$). PCC GSH/Cr was not significantly associated with total Yale-Brown Obsessive-Compulsive Scale score in the OCD group ($\beta = 5.7 \times 10^{-4}$; 95% confidence interval: -4.8×10^{-3} to 5.9×10^{-3}; $p = .83$).

CONCLUSIONS: Lower PCC GSH/Cr may be indicative of increased oxidative stress secondary to hypermetabolism in this brain region in OCD. Future MRS studies are warranted to investigate GSH levels in other brain regions that comprise the cortico-striato-thalamo-cortical circuit thought to be abnormal in OCD.

Keywords: Glutathione, MRS, Obsessive-compulsive disorder, OCD, Oxidative stress, Posterior cingulate cortex

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Obsessive-compulsive disorder (OCD), trichotillomania, excoriation disorder, and other pathological grooming disorders such as nail biting, lip biting, and cheek chewing are grouped by the DSM-5 (1) within a family of obsessive-compulsive and related disorders (OCRDs). Despite substantial prior research into these conditions, their pathogenesis remains unclear. Existing treatments are often only partially effective and these conditions may become chronic, resulting in reduced quality of life and often substantial morbidity (2). A better understanding of the underlying pathophysiology of these disorders could yield more efficacious treatments.

Several lines of evidence suggest that lower cerebral levels of glutathione (GSH), a key antioxidant in brain and other tissues (3), and/or increased oxidative stress contribute to OCRDs. First, polymorphisms of genes related to GSH regulation and oxidative stress have been associated with OCRDs. These include *SLC1A1*, which encodes the neuronal glutamate transporter (4)—the primary means of neuronal cysteine uptake and GSH production (5), and the mitochondrial genes encoding the proteins superoxide dismutase and uncouple-2, which buffer mitochondrial oxidative stress (6). Second, *N*-acetylcysteine (NAC), a potent antioxidant and precursor of GSH, has demonstrated efficacy in several OCRDs, including OCD, trichotillomania, and excoriation disorder (7). Third, levels of peripheral biomarkers for oxidative stress, including superoxide dismutase and malondialdehyde, a reactive oxygen species (ROS) and end product of lipid peroxidation, as well as GSH, are abnormal in OCD patients (6,8–13) and have been associated with symptom severity (9). Fourth, imaging studies report increased striatal levels of synaptic dopamine in OCD (14–18), which can be oxidized rapidly to ROS (19), thereby increasing oxidative stress within a key node of the cortico-striato-thalamo-cortical circuit thought to be abnormal in these disorders (20). Moreover, neuroimaging studies of people with OCD report higher striatal dopamine transporter densities (21,22), which could increase intracellular dopamine and levels of ROS, thus augmenting oxidative stress (23). Despite this evidence, no studies have used magnetic resonance spectroscopy (MRS) to investigate cerebral GSH levels in individuals with OCD, primarily because prior MRS studies of OCD employed MRS sequences that were not optimized for GSH quantification (24).

We recently completed a study assessing two-dimensional (2D) J-resolved proton MRS data from a pregenual anterior cingulate cortex (pgACC) voxel in individuals with OCD compared with age- and sex-matched non-OCD individuals (25).

The study found no group differences in any metabolites, including GSH, in the pgACC. Notably, this study generated additional MRS data, not included in our primary analyses, from a second voxel positioned over the posterior cingulate cortex (PCC)—a brain region evidencing structural (26), functional (27–29), and connectivity (30) abnormalities in prior studies of OCD individuals. Given evidence that individuals with OCD display PCC hyperactivation or hypermetabolism both at rest (31) and during cognitive challenge paradigms (28,29), which is attenuated by selective serotonin reuptake inhibitors (SSRIs) (31) and predicts clinical response to SSRIs (32) and anterior cingulotomy (33), we hypothesized that individuals with OCD would have lower GSH levels in PCC than non-OCD comparison individuals. This hypothesis was further stimulated by a recent finding in our center that striatal GSH levels are decreased in the *Sapap3* knockout mouse model of OCRDs (34). To test this hypothesis, we compared PCC GSH/total creatine (Cr) ratios in individuals with OCD versus those without OCD.

METHODS AND MATERIALS

Participant Selection

Participants age 18 years or older, right-handed, with a DSM-IV primary diagnosis of OCD, and scoring ≥18 on the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) were recruited from the Obsessive-Compulsive Disorders Institute at McLean Hospital, an intensive residential treatment program for severe OCD. Participants received magnetic resonance imaging (MRI) scans within 2 to 4 days of admission and before medication changes or initiation of behavioral therapy. Exclusion criteria included history of schizophrenia, bipolar disorder, Tourette syndrome, or an autism spectrum disorder; substance abuse or dependence (with the exception of nicotine) within 3 months of enrollment; primary hoarding as the primary OCD symptom; significant neurologic or medical illness; current pregnancy or lactation; or MRI contraindications. Comorbid mental disorders such as other OCRDs, major depression, and anxiety disorders were permitted, provided that they were not the primary presenting disorder. Since our original study was designed primarily to examine glutamate-related metabolites in the pgACC, participants taking medications affecting the glutamate system (e.g., memantine, riluzole, lamotrigine, topiramate, and NAC) were excluded. Other psychiatric medications were permitted if participants had received a stable dose ≥4 weeks before scanning.

Community individuals without OCD, age 18 years or greater, right-handed, with no DSM-IV psychiatric diagnosis, taking no psychoactive medications, and with no psychiatric illness among first-degree relatives were recruited by advertising. Exclusion criteria included positive urine screen for drugs of abuse before MRI scan, significant neurologic or medical illness, current pregnancy or lactation, or MRI contraindications.

Clinical Evaluation

After participants provided informed consent, approved by the McLean Hospital Institutional Review Board, we obtained demographic information, medical/psychiatric history, diagnoses via the Structured Clinical Interview for DSM-IV, OCD symptom information via the Y-BOCS, OCD symptom severity via the Y-BOCS, mood symptom severity via the Montgomery-Åsberg Depression Rating Scale, and anxiety symptom severity via the Hamilton Anxiety Rating Scale.

Image Acquisition

Imaging was conducted on a Siemens Trio 3-tesla scanner (Siemens Medical Solutions USA Inc., Malvern, Pennsylvania) at the McLean Imaging Center with a 32-channel Trans-Imaging Matrix platform upgrade. All participants underwent a routine anatomic MRI scan to screen for structural abnormalities and to guide ^{1}H-MRS voxel placement.

MRS acquisition and data analysis used a modified ^{1}H-MRS protocol similar to that described in previous studies from our group (25,35–38). Briefly, following acquisition of scout images, a 2 × 2 × 2 cm voxel was placed midsagittal, posterior to the splenium of the corpus callosum, and encompassing both dorsal and ventral subregions of the PCC (39), as well as portions of the precuneus and occipital lobe (Figure 1). Shimming of the magnetic field within the prescribed voxel was done using a machine-automated shimming routine. Following the additional automated optimization of water suppression power, carrier frequency, tip angles, and coil tuning, a modified J-resolved protocol (2D-JPRESS) was used based on prior experience demonstrating reliable fitting of GSH using this method (36). The 2D-JPRESS sequence collected 22 echo time (TE)-stepped spectra with the echo time ranging from 35 ms to 350 ms in 15-ms increments (repetition time = 2 seconds, f1 acquisition bandwidth = 67 Hz, spectral bandwidth = 2 kHz, readout duration = 512 ms, number of excitations = 16/TE-step, approximate scan duration = 12 minutes) providing enough J-resolved bandwidth (67 Hz) to resolve GSH, as well as other metabolites of interest including glutamate and glutamine (Figure 1 and Figure S1 in Supplement 1).

MRS Data Processing and Quantification

Spectroscopic data processing and analysis were undertaken on a LINUX workstation. To quantify GSH with the 2D-JPRESS data, the 22 TE-stepped, free-induction decay series was zero-filled out to 64 points, Gaussian-filtered, and Fourier-transformed using a GAMMA-simulated J-resolved basis set modeled for 3-tesla. Every J-resolved spectral extraction within a bandwidth of 67 Hz was fit with the spectral-fitting package LCModel (40) and its theoretically correct template—a method that we have used successfully in prior studies (25,35,36,38). The integrated area under the entire 2D surface for each metabolite was calculated by summing the raw peak areas across all 64 J-resolved extractions for each metabolite as described in our previous work (25,36,38). GSH levels are expressed as a ratio to total Cr.

Tissue segmentation of the PCC voxel was performed to rule out systematic differences in the percentage contribution of gray matter, white matter, or cerebrospinal fluid. Specifically, three-dimensional magnetization-prepared rapid acquisition gradient-echo axial image datasets were converted into NIfTI binary image file format using the FMRIB Software Library (FMRIB Analysis Group, Oxford University, Oxford, United Kingdom). FMRIB's Automated Segmentation Tool (FAST) was used for tissue segmentation of the T1-weighted image sets into gray matter, white matter, and cerebrospinal