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Grey matter changes associated with host genetic variation and exposure to Herpes Simplex Virus 1 (HSV1) in first episode schizophrenia

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ABSTRACT

Background: We previously reported reduced prefrontal cortex (PFC) grey matter volume among first episode, antipsychotic-naïve schizophrenia subjects (SZ) exposed to HSV1 but not among healthy subjects (HS) (Prasad et al., 2007). Independently, rs1051788, an exonic polymorphism of the MHC Class I polypeptide-related sequence B (*MICB*) gene was associated with HSV1 seropositivity, as well as SZ risk. In this study, we examined whether PFC grey matter changes associated with HSV1 exposure varied against the background of *MICB* genotypes.

Methods: We examined Caucasian individuals from the sample we studied in our previous report (Prasad et al., 2007) (SZ, $n=21$ and HS, $n=19$). Whole brain voxelwise analysis of structural MRI scans was conducted using Statistical Parametric Mapping, ver 5 (SPM5). The impact of rs1051788 variation and HSV1 seropositivity on grey matter volumes was examined using regression models on the combined sample of cases and controls, and then within each diagnostic group.

Results: In the combined sample of cases and controls, we observed the main effects of HSV1 seropositivity and genotypes, and a significant joint effect of HSV1 seropositivity and genotype mainly in the PFC. The joint effect was more prominent among cases than among controls.

Discussion: Our observations suggest that rs1051788 and HSV1 seropositivity are associated individually and jointly with reduced PFC grey matter volume. The patterns of these associations differ by diagnostic status, and these factors explain only a "small" portion of the variance in the grey matter volume reductions.

1. Introduction

Up to 90% of persons in the US depending on age, socioeconomic status and ethnicity are exposed to the Herpes Simplex Virus type 1 (HSV1) — a neurotropic virus (NHANES II 1987, revised 2007; Schillinger et al., 2004; Xu et al., 2006). The majority of such individuals do not develop encephalitis. Recent replicable evidence indicates that the serological evidence of exposure to HSV1 (HSV1 seropositivity) is associated with cognitive impairments in such individuals (Dickerson et al., 2003; Shirts et al., 2008a). The effect size of HSV1 exposure on cognitive impairment was greater among subjects with schizophrenia (SZ) compared to subjects without psychiatric disorders (Dickerson et al., 2008, 2003; Shirts et al., 2008a). Recently, we observed reduced prefrontal cortex (PFC) grey matter volumes among SZ subjects with serological evidence of exposure to HSV1 compared to those

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without but not among healthy subjects (HS) suggesting a mechanistic association that requires confirmation (Prasad et al., 2007). We had hypothesized that HSV1 exposure may interact with other illness-related variables such as genetic variations.

We have separately analyzed host genetic variations in association with HSV1 seropositivity in our association studies of SZ. We focused on the Human Leukocyte Antigen (HLA) region because it is frequently associated with susceptibility to infectious diseases. Following a series of genetic association studies (Kim et al., 2007; Shirts et al., 2007), we focused on the Major Histocompatibility Complex (MHC) Class I polypeptide-related sequence B (*MICB*) gene. Our analyses of rs1051788, an exonic polymorphism of *MICB* suggested novel associations. Allele A of this single nucleotide polymorphism (SNP) was associated with increased risk for SZ, whereas allele G was associated with elevated HSV1 antibody titers among HS, suggesting a pleiotropic effect (Shirts et al., 2007). This polymorphism leads to substitution of aspartic acid with asparagine at amino acid position 136 on one of the α-helical folds of MICB protein, suggesting that the substitution may be functionally relevant.

In view of the novel associations in our prior imaging and genetic association studies, we examined grey matter variations related to HSV1 exposure among first episode antipsychotic naïve SZ subjects and HS against the background of rs1051788 genotypes at *MICB*. Our goal in this study was to examine whether our previously observed differences in grey matter variations in the PFC among HSV1-exposed SZ subjects and HS is related to variation at a specific *MICB* polymorphism.

2. Methods

This sample was derived from our prior study, in which we examined associations between HSV1 exposure and grey matter volumes in first episode antipsychotic naïve SZ subjects and HS (Prasad et al., 2007). From this sample, we selected individuals with Caucasian ancestry in order to enable genetic analyses in a more ethnically homogenous sample ($n=40$; 21 first episode, antipsychotic-naïve SZ or schizoaffective disorder subjects, and 19 HS). The subjects were recruited from inpatient and outpatient services of the Western Psychiatric Institute and Clinic Pittsburgh, PA. DSM IV (American Psychiatric Association, 1994) diagnoses were derived by reviewing data from the Structured Clinical Interview for DSM IV diagnosis (SCID) (First, 1997) and medical records in a consensus diagnostic conference of senior clinicians. All diagnoses were reviewed after following the subjects for a minimum of 6 months. We also obtained demographic information, including the socioeconomic status (SES) using the Hollingshead scale (Hollingshead, 1975). HS were recruited through local advertisements from the same geographic region as the patients. None of the subjects had mental retardation, substance dependence in the last 6 months or abuse in the month before participation per DSM IV criteria. Subjects with medical or neurological disorders including head injury, encephalitis and epilepsy were not recruited. After fully explaining the experimental procedures, informed consents were obtained from all subjects. The Institutional Review Board of the University of Pittsburgh approved the study.

2.1. Imaging methods

MRI scans were obtained with a GE 1.5T whole body scanner (GE Medical Systems, Milwaukee, Wisconsin). The detailed scanning protocol has been described in our earlier publication (Gilbert et al., 2001). Briefly, the scans were three-dimension spoiled gradient recalled (SPGR), acquired in a steady-state pulse sequence (124 coronal slices, 1.5 mm thickness, TE = 5 ms, TR = 25 ms, acquisition matrix = 256 × 192, FOV = 24 cm, flip angle 40°). None of the scans had motion artifacts.

Images were analyzed using the Statistical Parametric Mapping (SPM 5) software (http://www.fil.ion.ucl.ac.uk/spm/software/spm5/). The voxel-based morphometry (VBM) analysis was implemented on a Mac Pro workstation using the SPM5 package on Matlab 7 platform (Mathworks Inc, 2002). SPM5 offers several advantages over the previous version such as updated segmentation procedure and correction for misclassified non-brain tissue. It reduces the reliance on template brain to perform these steps. The MR-images were normalized to the standard T1 template and segmented into grey matter, white matter and cerebrospinal fluid (CSF) depending on the intensity and the spatial probability of that voxel belonging to any of these tissue categories. The images were smoothed by convolving with a Gaussian smoothing kernel (12 × 12 × 12 FWHM) in order to reduce noise. The normalization procedure was reapplied to the original images to obtain optimized segmentation of normalized images. In order to correct for volume changes, segmented images were modulated by the Jacobian determinants from spatial normalization. All images were then smoothed with a 12 × 12 × 12 FWHM Gaussian kernel.

We first examined the grey matter density setting a significance threshold of uncorrected $p<0.001$ and an extent threshold of 20 voxels. We report only those clusters that remained significant after correction using the false discovery rate (FDR) method in the whole brain analyses. From these clusters of grey matter densities, we extracted the volumes from the native space using MarsBar (Brett et al., 2002) from all images used in that particular contrast. For example, in a contrast that compared HSV1 seropositive SZ subjects with HSV1 seronegative SZ subjects (HSV1+<HSV1−), the significant cluster that showed reduction in a given Brodmann area among HSV1 seropositive SZ subjects area was masked and the volume estimated from the native space of the images from HSV1 seropositive group; the corresponding volume from the native space of images from seronegative SZ subjects was also estimated using the same mask. The extracted volumes were compared using appropriate statistics on SPSS version 16 (SPSS, 2005). When a cluster consisted of multiple peaks of maximum intensity projections of grey matter density corresponding to a single Brodmann area, we added up the extracted volumes from these clusters and used this composite volume in the SPSS package for additional statistical tests as stated earlier. In order to locate the clusters to the Brodmann areas they correspond to, we converted the MNI coordinates to Talairach coordinates before using the Talairach database. We preferred to use the voxel-based