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Regulation of cortical and peripheral *GCH1* expression and biopterin levels in schizophrenia-spectrum disorders

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ABSTRACT

Tetrahydrobiopterin (BH_4) is an essential cofactor for dopamine, serotonin and nitric oxide synthesis. Deficits of plasma total biopterin (a measure of BH_4) have been described in schizophrenia and schizoaffective disorder. *GCH1* encodes the first and rate-limiting enzyme in BH_4 synthesis. Peripheral *GCH1* expression is lower in first episode psychosis patients versus controls, and we hypothesized that a *GCH1* promoter polymorphism associated with psychiatric illness, contributes to regulation of both *GCH1* expression and BH_4 levels. We tested this hypothesis in 120 subjects (85 patients with schizophrenia or schizoaffective disorder and 35 controls): Patients with the rs10137071 A allele had significantly lower plasma biopterin than GG patients and controls. In additional samples we assessed the relationship between genotype and diagnosis (schizophrenia or control) on *GCH1* expression in the prefrontal cortex ($n = 67$) and peripheral leukocytes ($n = 53$). We found a significant linear relationship between *GCH1* and study group in the CNS and periphery, with A allele patients having lower expression. Finally, in antipsychotic naïve patients ($n = 13$) we tested for an effect of medication on *GCH1*: Expression rose significantly after the onset of medication, primarily in A allele patients. These data suggest the potential for personalized genetic approaches to ameliorating BH_4 deficits in schizophrenia-spectrum disorders.

1. Introduction

In vivo synthesis of the monoamine neurotransmitters, dopamine (DA), serotonin (5-HT) and noradrenaline requires the enzyme cofactor tetrahydrobiopterin (BH_4) (Sumi-Ichinose et al., 2001; Thony et al., 2000). BH_4 is also vital for synthesis of nitric oxide (NO) by nitric oxide synthases (NOS), and stimulates and modulates the glutamatergic system (Koshimura et al., 1993; Mataga et al., 1991; Sumi-Ichinose et al., 2001; Thony et al., 2000). In the central nervous system (CNS), BH_4 has also been shown to stimulate the release of DA, 5-HT and glutamate, as well as regulating tyrosine hydroxylase protein levels at nerve terminals (Koshimura et al., 1990, 1994; Mataga et al., 1991; Sumi-Ichinose et al., 2001; Watanabe et al., 1991).

As dysregulation of amine neurotransmitters, glutamatergic systems and NO activity have all been implicated in the etiology of schizophrenia and affective disorders (Goff and Coyle, 2001; Lieberman et al., 1998; Meltzer et al., 2003; Shinkai et al., 2002; van Kammen and Kelley, 1991; Xing et al., 2002), and because CNS and peripheral biopterin levels are highly correlated (Richardson et al., 2005), in prior studies we measured levels of plasma total biopterin (a measure of BH_4, as approximately 80–90% of total biopterin are in the BH_4 form (Fiege et al., 2004; Kase et al., 2005). We observed significantly lower total biopterin levels in psychiatric patients, defining a mean plasma biopterin deficit of 34% in a large sample of schizophrenia patients (Richardson et al., 2005), and a mean deficit of 25% in schizoaffective disorder patients (Richardson et al., 2007), when compared to healthy control subjects. These significant biopterin deficits were observed after partialling out the effects of potential confounds including gender, age, ethnicity, neuroleptic use history and current dose, plasma phenylalanine (Phe) which stimulates BH_4 synthesis, and 24-h dietary Phe/protein ratio (relevant to BH_4 synthesis) (Richardson et al., 2005). A previous study of urine biopterin excretion showed no elevation in schizophrenia patients when compared to controls (Duch et al., 1984), suggesting that the plasma biopterin deficits result from reduced BH_4 synthesis rather than increased urine excretion. BH_4 deficits result in reduced levels of CNS amine neurotransmitters (reviewed in (Richardson et al., 2005)).

The rate-limiting and initial step in the de novo BH_4 synthesis pathway is catalyzed by the enzyme GTP cyclohydrolase I (GTPCH (EC 3.5.4.16)) encoded by the *GCH1* gene, which maps to chromosome

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14q22.1–22.2 (Gutlich et al., 1994; Thony et al., 2000). Levels of BH_4 and biopterin assayed in cells and tissues have been found to positively correlate with *GCH1* mRNA expression level (Tatham et al., 2009; Werner-Felmayer et al., 2002), and both biopterin level and *GCH1* mRNA level are markers of BH_4 biosynthesis. *GCH1* is thus a candidate for having an etiological role in psychiatric illness, via its regulation of BH_4, and this potential has been further supported by Kealey *et al.*, who showed association of a *GCH1* gene promoter polymorphism (NCBI dbSNP rs10137071 G/A polymorphism) with bipolar disorder (Kealey et al., 2005). More recently, lower peripheral blood leukocyte *GCH1* expression was demonstrated in first episode patients (FEP) with psychotic disorders (59% schizophrenia, 21% schizophreniform disorders, 13% brief psychotic disorders and 6% psychotic disorders NOS) as compared to controls (Ota et al., 2014). The importance of the *GCH1* promotor in regulating expression in psychiatric disorders was further suggested by the finding of increased methylation in FEP as compared to controls (Ota et al., 2014).

Based upon the association of a *GCH1* promotor polymorphism and lower peripheral expression with psychiatric illnesses, and increased promotor methylation in psychosis patients, we hypothesized that differences in variant-specific *GCH1* expression may contribute to our previously measured biopterin deficits. We therefore tested for effects of the rs10137071 variant on plasma biopterin levels in schizophrenia and schizoaffective disorder patients. Next, we measured *GCH1* expression in the CNS and peripheral blood leukocytes, testing for an effect of diagnosis and rs10137071 allele. Finally, in a group of never-medicated, neuroleptic naïve patients, we tested for an effect of antipsychotic medication on *GCH1* expression level (pre- to post-medication). Taken together, these data suggest the potential for personalized approaches to ameliorating BH_4 deficits.

2. Methods

2.1. Subjects

2.1.1. The biopterin sample

The Biopterin sample was obtained from a subsample of patients and control subjects previously described (Richardson et al., 2007, 2005). Subjects were included in this current study if they had a lifetime psychiatric diagnosis of schizophrenia or schizoaffective disorder (for patients), plus both a measure of fasting biopterin and a DNA sample for genotyping ($n = 120$ in total: $n = 85$ patients and $n = 35$ control subjects, see Table 1). Two subjects did not have a measure of total Phe, and thus $n = 118$ subjects were used in covariate analyses.

2.1.2. The Stanley Medical Research Institute (SMRI) array and neuropathology consortium collections

To investigate CNS *GCH1* expression in schizophrenia, postmortem brain tissue was donated from the (SMRI) brain collections; consisting of tissue blocks and/or extracted nucleic acids/protein collected from independent cohorts of psychiatric patients and controls (Stanley Medical Research Institute, 2014). To investigate prefrontal expression, we obtained RNA and DNA extracts from the SMRI's Array Collection: A collection of nucleic acids extracted from the dorsolateral prefrontal cortex (DLPFC) (Brodmann's area BA9/BA46) from Caucasian patients and controls, 75% of whom were male. We obtained samples from 67 subjects: 33 control subjects and 34 schizophrenia patients (RNA from two controls in the collection were missing, as was DNA from one patient).

We also received frozen tissue sections from the SMRI's Neuropathology Consortium Collection: Tissue from 15 control subjects and 14 schizophrenia patients from three regions including BA6 (premotor/ supplementary motor cortex), BA4 (primary motor cortex) and BA7 (parietal cortex). RNA and DNA was extracted from the Neuropathology Consortium Collection tissue blocks, as described below.

2.1.3. The leukocyte collections

To investigate *GCH1* expression in peripheral blood leukocytes, we recruited two samples, the first a sample of 28 male inpatients and 28 male controls. Patients were recruited from Rockland Psychiatric Center, Orangeburg, NY (the site of recruitment of our original Biopterin sample (Richardson et al., 2005)), and its associated outpatient facilities. A Structured Clinical Interview for DSM IV Disorders (SCID) interview was conducted for all patients to confirm a diagnosis of schizophrenia. The control subjects were recruited from the local hospital community. All controls completed a SCID non-patient interview. To further study *GCH1* expression in leukocytes, the second sample consisted of antipsychotic naive male patients ($n = 18$), recruited from the Comprehensive Psychiatric Emergency Program (CPEP) at Bellevue Hospital Center. Although recruitment was targeted for patients with schizophrenia, a SCID interview established that first-episode patients with schizoaffective disorder, bipolar disorder, major depression and delusional disorder, were also recruited. A sub-set of these patients ($n = 13$) participated in a follow-up visit approximately 1-week after the initiation of antipsychotic medication (10 received risperidone, 3 received aripiprazole). For these subjects, symptoms were assessed at both visits using the Brief Psychiatric Rating Scale (BPRS), the Schedule for Assessment of Positive Symptoms (SAPS), the Schedule for Assessment of Negative Symptoms (SANS) and the Mini-Mental State Examination (MMSE). For all these subjects (n = 28 inpatients; n = 25 control subjects; n = 18 antipsychotic naïve patients), bloods were collected at their study visit(s) and immediately processed for downstream expression analysis, as described below.

Written informed consent was obtained from all recruited subjects in accordance with the guidelines and regulations of the Nathan Kline Institute, New York University Langone Medical Center, and Columbia University Medical Center institutional review boards, and in accordance with New York State Office of Mental Health regulations and New York City Health and Hospitals Corporation regulations.

2.2. Study assays

2.2.1. Plasma biopterin and phe

Sample preparation and assay for plasma total biopterin has previously been reported (Richardson et al., 2005). Measurement of plasma Phe (assayed due to its known role in the regulation of BH_4 biosynthesis) has also been reported (Richardson et al., 2005).

2.2.2. Genotyping of the rs10137071 variant

Genomic DNA was extracted from whole blood or CNS tissue using standard methods (Qiagen Inc., Valencia, CA, USA). Primers were utilized to amplify a 351 bp genomic region spanning the G/A variant as described previously (Kealey et al., 2005). The restriction enzyme BsrD1 recognizes and cleaves the A allele resulting in the formation of a 191/160 bp doublet.

2.2.3. GCH1 Expression in the CNS via quantitative RT-PCR

For the SMRI Neuropathology Consortium Collection, RNA was extracted from 150 mg frozen tissue using a standard trizol procedure, followed by a DNAse I treatment and purification using the RNeasy MiniElute Kit (Qiagen Inc., Valencia, CA). For both the Array and Neuropathology Consortium Collections, 200 ng of RNA was employed for first strand cDNA synthesis, in a 20ul reaction volume using a 1:1 ratio of random hexamer and oligo-dT primers and Superscript® III RT enzyme (Life Technologies Inc., Carlsbad, CA). cDNA products were diluted with 80ul RNAse free water, prior to quantitative PCR of *GCH1*, plus the housekeeping genes *B2M* and *GAPDH* for normalization. PCRs were performed by monitoring in real time the increase in fluorescence using TaqMan™ Gene Expression Assays (Thermo Fisher Scientific, Waltham, MA), using a Bio-Rad iQ5 detector system (Bio-Rad, Hercules, CA). Briefly, 8ul of the cDNA product was employed in triplicate 20ul PCR duplexed reactions including the FAM-labeled GCH1 Taqman