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Evidence for association of hyperprolinemia with schizophrenia and a measure of clinical outcome

Catherine L. Clelland [a], Laura L. Read [b,c], Amanda N. Baraldi [c], Corinne P. Bart [c], Carrie A. Pappas [c], Laura J. Panek [c], Robert H. Nadrich [b,d], James D. Clelland [b,c,*]

[a] Department of Pathology and Cell Biology, and Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University Medical Center, 630 West 168th Street, New York, NY, United States
[b] Department of Psychiatry, New York University Langone Medical Center, 550 First Avenue, New York, NY, United States
[c] Movement Disorders and Molecular Psychiatry, The Nathan Kline Institute for Psychiatric Research, 140 Old Orangeburg Road, Orangeburg, NY, United States
[d] Bellevue Hospital Center, 462 First Avenue, New York, NY, United States

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ABSTRACT

There are multiple genetic links between schizophrenia and a deficit of proline dehydrogenase (PRODH) enzyme activity. However, reports testing for an association of schizophrenia with the resulting proline elevation have been conflicting. The objectives of this study were to investigate whether hyperprolinemia is associated with schizophrenia, and to measure the relationship between plasma proline, and clinical features and symptoms of schizophrenia.

We performed a cross-sectional case–control study, comparing fasting plasma proline in 90 control subjects and 64 schizophrenic patients and testing for association of mild to moderate hyperprolinemia with schizophrenia. As secondary analyses, the relationship between hyperprolinemia and five measures of clinical onset, symptoms and outcome were investigated.

Patients had significantly higher plasma proline than matched controls ($p<0.0001$), and categorical analysis of gender adjusted hyperprolinemia showed a significant association with schizophrenia (OR 6.15, $p=0.0003$). Hyperprolinemic patients were significantly older at their first hospitalization ($p=0.015$ following correction for multiple testing). While plasma proline level was not related to total, positive or negative symptoms, hyperprolinemic status had a significant effect on length of hospital stay ($p=0.005$), following adjustment for race, BPRS score, and cross-sectional time from admission to proline measurement. Mild to moderate hyperprolinemia is a significant risk factor for schizophrenia, and may represent an intermediate phenotype in the disease. Hyperprolinemic patients have a significantly later age of first psychiatric hospitalization, suggestive of later onset, and hospital stays 46% longer than non-hyperprolinemic subjects. These findings have implications in the etiology of schizophrenia, and for the clinical management of these patients.

1. Introduction

Schizophrenia is a severe psychiatric disorder of unknown cause, with a worldwide incidence of approximately 1%. There is a large increased risk of schizophrenia and other psychotic disorders in people with 22q11 deletion syndrome (22q11DS), with up to one third developing schizophrenia or schizoaffective disorder (Murphy et al., 1999; Scambler, 2000; Jacquet et al., 2002; Karayiorgou and Gogos, 2004; Karayiorgou et al., 2010). A common feature of 22q11DS is a hemizygous deletion of the proline dehydrogenase (PRODH) gene, which encodes the proline dehydrogenase enzyme, that catalyzes the first step in proline catabolism (Mitsubuchi et al., 2008). Significantly, approximately 37–50% of patients with the 22q11 deletion (Goodman et al., 2000; Raux et al., 2007) have elevation of plasma proline that is between 2 and 10 fold higher than the upper end of the normal range (Mitsubuchi et al., 2008), and plasma proline levels have been found to inversely correlate with intelligence quotient in patients with the 22q11DS velo-cardiofacial syndrome (Raux et al., 2007).

In addition to its proteogenic role, proline is a precursor of the neurotransmitter glutamate, and has several characteristics that suggest it functions as a CNS neuromodulator (Phang et al., 2001). Studies of elevated proline in humans and model systems illustrate some of the pathogenic properties of hyperprolinemia: In the hyperprolinemic PRO/RE mouse strain, elevated peripheral and CNS proline are associated with neurocognitive dysfunction, in the form of learning and memory deficits (Baxter et al., 1985; Davis et al., 1987). Deficiency of PRODH activity in the PRO/RE mouse, which results from

* Corresponding author at: Movement Disorders and Molecular Psychiatry, The Nathan Kline Institute for Psychiatric Research, 140 Old Orangeburg Road, Orangeburg, NY 10962, United States. Tel.: +1 845 398 5512; fax: +1 845 398 5518.
E-mail address: clelland@nki.rfmh.org (J.D. Clelland).

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a heterozygous nonsense Prodh mutation (the premature termination E453X variant (Gogos et al., 1999)), closely mimics the loss of PRODH activity and the 2–10 fold elevation of plasma proline observed in human hyperprolinemia type-I (HPI), which also arises from mutations in the PRODH gene (Mitsubuchi et al., 2008). Although variable, the neurological phenotype associated with HPI includes mental retardation and epilepsy (Afenjar et al., 2007; Mitsubuchi et al., 2008). Plasma proline elevations greater than 10-fold above the normal range are found in patients with hyperprolinemia type-II (HPII), caused by mutations in the ALDH4A1 gene that encodes Δ-1-pyrroline-5-carboxylate (P5C) dehydrogenase, which is immediately downstream of PRODH in proline catabolism. P5C dehydrogenase deficits and the resultant hyperprolinemia can lead to low IQ, seizures, and in some subjects, mild mental retardation (Flynn et al., 1989). Following chronic proline administration, rats with plasma proline levels consistent with human HPII developed behavioral and brain histological changes coupled with impairments of glutamate synthesis, all suggestive of neurological dysfunction (Shanti et al., 2004).

Evidence supporting the functional significance of hyperprolinemia in schizophrenia comes from two sources: Mice homozygous for the Prodh E453X mutation, have elevated plasma and brain proline, locally decreased CNS glutamate and γ-aminobutyric acid (GABA) (Gogos et al., 1999; Paterlini et al., 2005), and a deficit in sensorimotor gating shown as decreased prepulse inhibition of the acoustic startle response, that is a characteristic of schizophrenia (Braff et al., 1978). Moreover, a familial PRODH deletion and PRODH missense mutations that have been described in patients with schizophrenia (Jacquet et al., 2002; Bender et al., 2005), and that have been functionally related to both moderately and severely decreased PRODH enzyme activity *in vitro* (Bender et al., 2005), have also been associated with both HPI and moderate hyperprolinemia in schizophrenic patients (Jacquet et al., 2002). However, the conclusions of case–control studies evaluating peripheral proline levels as a risk factor for schizophrenia have been conflicting: Following measurement of plasma proline, Jacquet et al. did not detect an association between mild to moderate hyperprolinemia and schizophrenia in a mixed-gender study of Caucasian subjects, although they did report hyperprolinemia as a significant risk for schizoaffective disorder (Jacquet et al., 2005). This study concurred with a previous report, finding no significant difference in serum proline level across groups of control subjects, treated schizophrenics, naive schizophrenics and drug-free schizophrenic subjects (Rao et al., 1990). Conversely, a more recent study also measuring serum levels found a significant elevation of proline in schizophrenic patients when compared to controls, but only in female subjects (Tomiya et al., 2007). Despite these mixed findings, data continues to support a functional role for PRODH variants and hyperprolinemia in the etiology of schizophrenia (Kempf et al., 2008), although studies relating plasma proline level to the clinical symptoms of schizophrenia are lacking. The objective of this study was to test the hypothesis that elevated peripheral proline is associated with schizophrenia after adjusting for gender differences, and to explore the clinical effects of elevated proline levels in schizophrenic patients.

2. Methods

2.1. Subjects and recruitment

Male and female, African American, Caucasian and Hispanic patients, aged 18–65, were recruited from inpatient wards at Bellevue Hospital Center (BHC). A significant effect of valproic acid (VPA) on plasma proline level was previously reported (Jacquet et al., 2005), and therefore schizophrenic subjects treated with VPA at the time of enrollment were excluded. Patient screening and recruitment was not dependent on their length of stay in the hospital at the time of recruitment, and thus cross-sectional data were generated. Patients received a standardized hospital diet based upon ADA Guidelines of 20% protein, 25% fat and 55% carbohydrates. Psychiatric symptoms were measured using the Brief Psychiatric Rating Scale (BPRS), the Schedule for Assessment of Positive Symptoms (SAPS), the Schedule for Assessment of Negative Symptoms (SANS), and schizophrenia diagnoses were confirmed using the Structured Clinical Interview for DSM IV Disorders (SCID).

Controls were recruited from the BHC community, with recruitment targeted to reflect the patients on age, race/ethnicity, and gender. A SCID-NP interview was conducted for all controls, who were excluded if they reported symptoms from modules A-D. All subjects completed general questionnaires, self-reporting race, and documenting diagnostic and medical history information for common diseases and prescription medication use. Capacity to give informed consent was determined in accordance with the New York University (NYU) IRB regulations. After description of the study to the subjects, written informed consent was obtained from all subjects in accordance with all institutional IRB guidelines and regulations.

2.2. Determination of plasma proline levels

For all subjects, a fasting morning blood draw was performed and heparinized blood samples sent to ARUP Laboratories (500 Chipeta Way, SLC, UT84108) for quantitative plasma amino acid analysis (reference number 0080710). Proline was measured in μmoles/liter (μM).

2.3. Statistical analysis

Group differences were tested using the Satterthwaite t-test or ANOVA with a correction for multiple testing (assuming normality of continuous variables), and using the χ^2 or Fisher exact test where the expected cell size was <5 (categorical variables).

Tests of normality ($n = 154$, $p < 0.001$) and inspection of the proline distribution suggested non-symmetry with a positive skew and heavier than normal tails. Therefore, proline levels were compared across groups using the Mann–Whitney and Kruskal–Wallis non-parametric tests, and the Spearman's rank correlation coefficient to assess relationships with continuous variables. To adjust for previously reported gender differences (Jacquet et al., 2005), Jacquet et al.'s criteria were employed to define hyperprolinemic status as a proline level two standard deviations (SDs) or more above the gender-specific mean of controls (Jacquet et al., 2005).

We sought to determine the effect of plasma proline on five clinical measures collected, using a generalized linear modeling (GLM) approach, employing a maximum-likelihood estimation to summarize the relationship between hyperprolinemia and the clinical outcomes of total BPRS, SAPS, and SANS scores, age at first hospitalization, and length of hospital stay (LOHS). To model LOHS, subjects were excluded from analysis if they were transferred to another treatment facility ($n = 19$), as discharge due to improvement could not be considered. Distributional assumptions were tested for each dependent variable using the Anderson–Darling test (Supplementary Data S1). For models that passed criteria (a relationship with hyperprolinemia when alpha <0.1), medication (CPZ equivalent daily dose), severity of illness (total BPRS, SAPS, and SANS scores), history of alcohol abuse/dependence, smoking status, prior housing status before admission, plus the demographic variables age, race, gender, current occupational status (currently working or attending school compared to those currently unemployed), and highest education level reached (excluding subjects still in education) were assessed as possible covariates. Due to the cross-sectional nature of the data collection, the variable of time from admission to proline measurement was also evaluated as a covariate in the LOHS model. To assess utility in adjusting the dependent variable, each covariate was entered into a bivariate analysis, and terms found to have p values of <0.10 carried forward to a multivariate model, where we examined the effect of plasma proline on LOHS while controlling for significant