Evidence that COMT genotype and proline interact on negative-symptom outcomes in schizophrenia and bipolar disorder

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Elevated peripheral proline is associated with psychiatric disorders, and there is evidence that proline is a neuromodulator. The proline dehydrogenase (PRODH) gene, which encodes the enzyme that catalyzes proline catabolism, maps to human chromosome 22q11.2, a region conferring risk of schizophrenia. In the Prodh-null mouse, an interaction between elevated peripheral proline and another 22q11.2 gene, catechol-O-methyltransferase (COMT), on neurotransmission and behavior has been reported. We explored the relationship between fasting plasma proline levels and COMT Val158Met genotype on symptoms (positive, negative and total) in schizophrenia patients. In an exploratory study we also examined symptom change in patients with bipolar disorder. There was a significant interaction between peripheral proline and COMT on negative symptoms in schizophrenia (P < 0.0001, n = 95). In COMT Val/Val patients, high proline was associated with low Scale for the Assessment of Negative Symptom (SANS) scores. In contrast, high proline was associated with high SANS scores in patients carrying a Met allele. The relationship between proline and COMT also appears to modify negative symptoms across psychiatric illness. In bipolar disorder, a significant interaction was also observed on negative-symptom change (P = 0.007, n = 43). Negative symptoms are intractable and largely unaddressed by current medications. These data indicate a significant interaction between peripheral proline and COMT genotype, influencing negative symptoms in schizophrenia and bipolar disorder. That high proline has converse effects on symptoms by COMT genotype, may have implications for therapeutic decisions.

Translational Psychiatry (2016) 6, e891; doi:10.1038/tp.2016.157; published online 13 September 2016

INTRODUCTION
Schizophrenia symptoms are typically divided into positive, negative and cognitive clusters, along with mood symptoms.1 Positive symptoms, including hallucinations and delusions, show the greatest response to treatment, whereas cognitive symptoms such as conceptual disorganization, and negative symptoms including avolition, blunted affect and social withdrawal, are largely unaddressed by current medications. Indeed, negative symptoms are among the most persistent and debilitating in schizophrenia,13 but also in the etiology of endophenotypes associated with schizophrenia.14

PRODH maps to chromosome 22q11.2, a region associated with the highest known genetic risk for schizophrenia, aside from that shared by monozygotic twins. In addition, this location is also associated with the hemizygous microdeletion found in 22q11.2 deletion syndrome (22q11DS), and there is an increased risk of schizophrenia as well as other psychotic, mood-, obsessive compulsive- and autism spectrum disorders in 22q11DS patients.15–19 Approximately 37–50% of 22q11DS patients have significant elevation of fasting plasma proline.20 The catechol-O-methyltransferase gene (COMT) encodes the enzyme that methylates and inactivates catecholamines including dopamine, and also maps to 22q11.2, distal to PRODH, the COMT Val158Met functional polymorphism (substitution of valine (Val) to methionine (Met) at residue 158), has been extensively studied with regards to dopamine neurotransmission, because Val/Val homozygotes have prefrontal cortical (PFC) enzyme activity ~40% higher than Met/Met homozygotes and are considered to have concomitant lower PFC dopamine levels.21,22 It has thus been suggested that the Val158Met polymorphism modulates cognitive functioning (reviewed in Bilder et al.23).

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Received 26 February 2016; revised 26 May 2016; accepted 12 July 2016
Although COMT has been associated with psychotic and mood disorders including schizophrenia and bipolar disorder,\textsuperscript{24,25} results have been inconsistent.\textsuperscript{26} A CNS functional interaction between COMT and PRODH has been proposed by Paterlini et al., who suggested that significant cortical Comt upregulation in the Prodh-null mouse represents a deficiency enhancing glutamatergic synaptic transmission.\textsuperscript{28} In addition, psychosis with activity Met allele. Given these reports, and our levels of plasma proline in 22q11DS patients carrying the low-

| Characteristics                  | Met/Met n = 21 | Val/Met n = 32 | Val/Val n = 42 | P-value* |
|----------------------------------|---------------|---------------|---------------|----------|
| Gender, n (%)                    |               |               |               | 0.288    |
| Female                           | 11 (23.4)     | 19 (40.4)     | 17 (36.2)     |          |
| Male                             | 10 (20.8)     | 13 (27.1)     | 25 (52.1)     |          |
| Ethnicity, n (%)                 |               |               |               | 0.096    |
| African American                 | 5 (13.5)      | 10 (27.0)     | 22 (59.5)     |          |
| Caucasian                        | 10 (35.7)     | 10 (35.7)     | 8 (28.6)      |          |
| Hispanic                         | 6 (20.0)      | 12 (40.0)     | 12 (40.0)     |          |
| Age (years), mean ± s.d.         | 40.9 ± 10.9   | 39.1 ± 11.5   | 39.9 ± 11.6   | 0.820    |
| History of alcoholism, n (%)     |               |               |               | 0.426    |
| Neither                          | 17 (23.6)     | 27 (37.5)     | 28 (38.9)     |          |
| Abuse                            | 1 (10.0)      | 2 (20.0)      | 7 (70.0)      |          |
| Dependence                       | 3 (23.1)      | 3 (23.1)      | 7 (53.8)      |          |
| Duration of Illness (years), b mean ± s.d. | 19.67 ± 12.62 | 13.11 ± 10.99 | 11.5 ± 10.23 | 0.136    |
| Hospital duration (days), c mean ± s.d. | 19.1 ± 17.1  | 21.9 ± 23.4   | 20.0 ± 19.6   | 0.998    |
| Fasting plasma proline, μmol l\(^{-1}\) | 219.9 ± 91.6 | 240.5 ± 68.6  | 246.4 ± 91.1  | 0.391    |
| Symptoms                         |               |               |               |          |
| BPRS\textsuperscript{4} total symptoms, mean ± s.d. | 32 ± 8.5      | 33.6 ± 7.1    | 33.6 ± 8.4   | 0.500    |
| SAPS\textsuperscript{5} total symptoms, mean ± s.d. | 10.3 ± 8.3    | 15.8 ± 9.6    | 18.2 ± 10.1  | 0.006\textsuperscript{6} |
| SANS\textsuperscript{7} total symptoms, mean ± s.d. | 24 ± 16.8     | 21.8 ± 13.1   | 17.5 ± 13.9  | 0.127    |
| Neuroleptic medications          |               |               |               | 0.348    |
| Neuroleptic type, n (%)           |               |               |               |          |
| Typical                          | 5 (27.8)      | 3 (16.7)      | 10 (55.6)     |          |
| Atypical                         | 13 (72.4)     | 19 (32.8)     | 26 (44.8)     |          |
| Both                             | 3 (16.7)      | 9 (50.0)      | 6 (33.3)      |          |
| Daily CPZE dose,\textsuperscript{9} mean ± s.d. | 490.6 ± 234.0 | 571.1 ± 418.1 | 526.8 ± 281.0 | 0.981    |
| Mood-stabilizing medications     |               |               |               |          |
| Mood stabilizer yes; n (%)        | 15 (26.3)     | 19 (33.3)     | 23 (40.4)     | 0.443    |
| VPA treatment yes; n (%)          | 4 (12.9)      | 11 (35.5)     | 16 (51.6)     | 0.327    |
| Other medications                |               |               |               |          |
| Benzodiazepines; yes; n (%)       | 4 (21.0)      | 8 (42.1)      | 7 (36.8)      | 0.641    |
| Antidepressants; yes; n (%)       | 1 (9.1)       | 5 (45.4)      | 5 (45.4)      | 0.596    |

Abbreviation: COMT, catechol-O-methyltransferase; CPZE, chlorpromazine. *Significant P-value when comparing characteristics across COMT genotypes. \textsuperscript{b}Determined as the period since the subjects’ first hospitalization for psychiatric symptoms, n = 60 for whom this characteristic could be obtained. \textsuperscript{c}Days in hospital prior to fasting blood draw. \textsuperscript{d}Brief Psychiatric Rating Scale. \textsuperscript{e}Schedule for Assessment of Positive Symptoms. \textsuperscript{f}Schedule for Assessment of Negative Symptoms. \textsuperscript{g}CPZE equivalent dose, n = 94 (as one subject’s NL had no CPZ equivalent).

Table 1. Demographics of schizophrenic sample, n = 95

Subjects
Schizophrenia and bipolar disorder patients, aged 18–65 years, were recruited from Bellevue Hospital Center (BHC), a primary care facility, servicing relatively short-stay inpatients with acute psychiatric needs. The diagnosis of all patients was confirmed using the Structured Clinical Interview for DSM IV Disorders. After description of the study to subjects, written informed consent was obtained in accordance with institutional review board regulations.

Demographics and group descriptive data for the schizophrenia sample are shown in Table 1. Although recruitment was not targeted by COMT, patients were well-matched across groups. For schizophrenia patients, recruitment was cross-sectional and independent of their duration of hospitalization. Psychiatric symptoms were measured using the Schedule
Table 2. Demographics of bipolar disorder sample, n = 43

| Characteristics | Met/Met n = 5 | Val/Met n = 22 | Val/Val n = 16 | P-value* |
|-----------------|--------------|---------------|--------------|----------|
| Gender, n (%)   |              |               |              |          |
| Female          | 1 (62)       | 11 (68.8)     | 4 (25.0)     | 0.328    |
| Male            | 4 (14.8)     | 11 (40.7)     | 12 (44.4)    |          |
| Ethnicity, n (%)|              |               |              |          |
| African American| 0 (4.57)     | 3 (42.9)      |              | 0.450    |
| Asian           | 0 (4.57)     | 1 (100)       |              |          |
| Caucasian       | 3 (11.5)     | 13 (50.0)     | 10 (38.5)    |          |
| Hispanic        | 2 (22.2)     | 5 (55.6)      | 2 (22.2)     |          |
| Age (years), mean ± s.d. | 34 ± 9.7 | 32.8 ± 8.4 | 33.2 ± 11.2 | 0.933 |
| History of Alcoholism, n (%) | 1.000 |
| Abuse           | 1 (7.1)      | 8 (57.1)      | 5 (35.7)     |          |
| Dependence      | 1 (12.5)     | 4 (50.0)      | 3 (37.5)     |          |
| Neither         | 3 (14.3)     | 10 (47.6)     | 8 (38.1)     |          |
| Fasting proline, μmol l⁻¹ | 213.6 ± 72.7 | 205.5 ± 63.2 | 245.8 ± 123.4 | 0.669 |
| Illness duration (years), mean ± s.d. | 5.5 ± 6.8 | 4.9 ± 6.0 | 9.3 ± 8.7 | 0.304 |
| Days between symptom assessments, mean ± s.d. | 10.2 ± 6.2 | 9.4 ± 3.7 | 9.5 ± 5.1 | 0.382 |

*P-value when comparing Met allele carriers to Val/Val patients. aSampled at visit 1. Determined as the period since the subjects' first hospitalization for psychiatric symptoms, n = 35 for whom this characteristic could be obtained.

RESULTS

COMT genotype modifies the relationship between proline and negative symptoms of schizophrenia

The schizophrenia sample (n = 95) was well-matched across genotype groups, and similar in size to previously published studies in 22q11DS reporting a significant COMT×proline interaction.27,28 There were no differences in BPRS total or negative symptoms (SANS total score, Table 1). As previously reported33 Met/Met patients had significantly lower SAPS scores than Val/Met (Mann–Whitney z = 2.52, adjusted P = 0.035) or Val/Val patients (z = 2.92, adjusted P = 0.001). We achieved 100% accuracy from confirmatory regenotyping and a sample of 90 control subjects were in Hardy–Weinberg equilibrium for COMT Val158Met (P > 0.05, data not shown). COMT distributions of the schizophrenia patients deviated from Hardy–Weinberg equilibrium (χ² = 8.08, df = 1, P < 0.05), which has been previously reported for this polymorphism in schizophrenia.84

Testing the primary hypothesis of an interaction effect on schizophrenia symptoms; results of the multivariate analysis of covariance for symptom scores showed a significant genotype×proline interaction, Wilk’s Λ = 0.69, F(2,174) = 6.01, P = 0.0001 (Supplementary Table 2). Follow-up Wald tests with Bonferroni correction identified significant interaction effects specific to total SANS scores F(2,89) = 13.33, adjusted P < 0.001 but no significant interaction effect on total SAPS F(2,89) = 2.97, adjusted P = 0.168, or BPRS scores F(2,89) = 0.42, adjusted P = 1.0, suggesting specificity of the relationship to negative symptoms.

To examine the significant multivariate analysis of covariance on total SANS scores, interaction effects were graphed in Figure 1a (Supplementary Figure 2). For schizophrenia patients with both the Met/Met and Val/Val genotypes, high proline was associated with high SANS scores, while conversely high proline in Val/Val patients was associated with lower-negative-symptom scores. Stratification by COMT allele carrier (for Met allele carrier or Val/Val), for Met carriers every 100 μM increase in proline (~1 s.d. from the mean proline level), was associated with a SANS total score increase of over 8 points (β coefficient = 0.084, P = 0.001). Conversely, for Val/Val patients every 100 μM increase in proline decreased SANS total scores by nearly 7 points (β = −0.067, P = 0.003). Thus, at proline levels only ~1 s.d. above the group means, Met carriers with a fasting plasma proline of 332 μM have a predicated SANS score of 30, whereas Val/Val patients with proline of 346 μM have a predicted score of only 10. The significant interaction remained

Statistical analysis

Demographic and clinical characteristics were compared across genotypes, using ANOVA, Kruskal–Wallis and Mann–Whitney tests, χ² or Fisher exact tests as appropriate. Genotype distributions were tested for Hardy–Weinberg equilibrium using an exact test.

Multivariate analysis of covariance was employed to test the hypothesis of an interaction effect between COMT genotype and the continuous predictor variable fasting plasma proline, on schizophrenia symptoms (total SANS, SAPS and BPRS scores). Estimates of the interaction coefficients were obtained from the multivariate regression model, and tested for significance across the three dependent variables, with Bonferroni correction for post hoc comparisons. Homogeneity of variance and covariance matrices assumptions were confirmed (P > 0.05) using Levine’s and Box-M tests, respectively. Specific significant interaction effects that remained were assessed further via multivariable regression. To evaluate and then control for potential confounds between demographic and/or clinical characteristics with the dependent variable(s), covariates were entered into a bivariate linear regression and terms found to have P-values < 0.10 carried forward to a multivariable model. Gender was a covariate in all models, to adjust for previously reported proline gender differences.27,28 Multivariable model fit and selection was determined via Wald tests, testing the null hypothesis that non-significant (P > 0.05) covariate parameters were simultaneously equal to zero in full and subsequent reduced model. On the basis of the result from the schizophrenia study, in an exploratory analysis the primary outcome for bipolar patients was the BPRS-negative-symptom subscale,27 and percent reduction in negative symptoms calculated (for bipolar disorder patients, the range of BPRS scores at the blood draw visit (range 5–8) did not allow for cross-sectional analysis). When outliers in the data or leverage points were identified, a robust regression procedure using an MM estimator to minimize data point effects (SASv9.3) was employed. All statistical tests were two-tailed. Means and s.d. are reported. Statistical analysis was performed in SASv9.3 and Stata ICv12, with graphs plotted in Ggplot2v1.0.1 in Rv3.1.2.
following stratified analysis of ethnicity, which was found not to influence the interaction effect (Supplementary Table 3), stratification and then adjustment for duration of illness (determined as the period since the subjects’ first hospitalization for psychiatric symptoms; Supplementary Table 4), and after removal of patients reporting alcohol abuse/dependence (P < 0.005, n = 72).

Supporting our SANS finding, we also observed a significant interaction between COMT (Met carriers versus Val/Val patients) and high proline on the negative-symptom subscale of the BPRS (interaction β = −0.0266, P = 0.002).

Possible confounds on the COMT × proline interaction on total SANS score were assessed. Covariate analysis showed no relationship between SANS score and medication type, neuroleptic dose (summarized as daily chlorpromazine equivalents), duration of illness or the number of days in hospital prior to blood draw and symptom assessment (Supplementary Table 5). However, there was a relationship between SANS and ethnicity and alcohol use (P < 0.1) and along with gender these variables were taken forward to a multivariable model (Table 3). Model fit was determined with the final model retaining genotype (ordinal due to the graphed relationship observed in Figure 1a), proline, alcohol use and the highly significant COMT-proline interaction (P < 0.0001).

Valproate-treated COMT Val/Val schizophrenia patients have significantly lower-negative symptoms than Met allele carriers. An effect of VPA on plasma proline has been reported31 and VPA-treated schizophrenia patients in our study had significantly higher proline (mean: 299.29 ± 94.76, n = 28) than those who did not receive VPA (mean: 215.84 ± 63, n = 64; z = −3.97, P = 0.0001). Considering our finding of an interaction between COMT and
proline on negative symptoms, we next hypothesized that VPA-treated Val/Val patients would respond differently to the concomitant higher levels of proline, with respect to their negative symptoms, as compared with Met carriers. As shown in Figure 1b, VPA-treated Val/Val schizophrenia patients had significantly lower SANS total scores, averaging 12 points lower than Val/Met and Met/Met patients ($\beta = -12.17, P = 0.041, n = 28$). This result remained significant after adjusting for the dose of VPA administered in the 48 h prior to the blood draw ($P = 0.043$).

**DISCUSSION**

The data presented in this study demonstrate that fasting peripheral proline and COMT Val$^{158}$Met genotype, predict negative-symptom severity in schizophrenia. Specifically, we present evidence that for inpatients with the Val/Val genotype (encoding the high-activity COMT enzyme), high proline was associated with lower levels of negative symptoms: as proline rose across the Val/Val patients, negative symptoms decreased. Conversely, Met allele carriers displayed the opposite relationship, exhibiting significantly more negative symptoms as proline levels rose. Over the range of fasting proline in our schizophrenia sample (87–502 $\mu$m), this represents a significant and clinically relevant difference in negative symptoms between COMT genotype groups.

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**Table 3.** Results: the interaction between proline and COMT predicts negative symptoms in schizophrenia

| Dependent Variable = Total SANS Score, $n = 95$ | $\beta$ coefficient | s.e. | Test statistic$^a$ | P-value | Wald test |
|-----------------------------------------------|---------------------|------|-------------------|---------|-----------|
| **Initial Model$^b$**                          |                     |      |                   |         |           |
| Proline                                       | -0.0870             | 0.0317| 2.25              | 0.0126* |           |
| COMT (ordinal Val/Val, Val/Met, Met/Met)      | -9.7339             | 4.0994| 1.19              | 0.2782* |           |
| Interaction (COMT x proline)                  | 0.0560              | 0.0167| 16.08             | 0.0000* |           |
| **Full Model$^b$**                             |                     |      |                   |         |           |
| Proline                                       | -0.1050             | 0.0333| 2.43              | 0.0150* |           |
| COMT (ordinal Val/Val, Val/Met, Met/Met)      | -13.0179            | 4.2452| 11.74             | 0.0008* |           |
| Interaction (COMT x proline)                  | 0.0744              | 0.0169| 5.61              | 0.0000* |           |
| Alcohol use                                   |                     |      |                   |         |           |
| Alcohol abuse vs none                         | -4.2178             | 4.2706| 1.02              | 0.3092* |           |
| Alcohol dependence vs none                   | -9.0807             | 3.5632| 2.56              | 0.0108* |           |
| **Gender**                                    |                     |      |                   |         |           |
| African American vs Caucasian                | 3.0258              | 3.0258| 1.27              | 0.2039* |           |
| African American vs Hispanic                  | 4.6703              | 2.8214| 6.49              | 0.0099  |           |
| **Final Model$^b$**                            |                     |      |                   |         |           |
| Proline                                       | -0.0804             | 0.0321| 6.28              | 0.0122* |           |
| COMT (ordinal Val/Val, Val/Met, Met/Met)      | -9.6576             | 4.0300| 1.85              | 0.0668* |           |
| Interaction (COMT x proline)                  | 0.0651              | 0.0161| 10.60             | 0.0000* |           |
| Alcohol use                                   |                     |      |                   |         |           |
| Alcohol abuse vs none                         | -5.1234             | 3.9854| 1.66              | 0.1986* |           |
| Alcohol dependence vs none                   | -9.7478             | 3.3526| 4.45              | 0.0036* |           |

Abbreviations: COMT, catechol-O-methyltransferase; SANS, Scale for the Assessment of Negative Symptom.$^a$ $\chi^2$ (Schizophrenia models using Robust linear regression). $^b$Robust regression, MM Estimation Method; $^c$ Robust Wald test: canonical linear hypothesis that combined effect of covariates (Gender and Ethnicity, bold) is zero. $^d$Robust Wald test: covariate effect (Alcohol use, italics) is zero.
VPA upregulates circulating proline and, in our study VPA-treated schizophrenia Val/Val patients had significantly less-negative symptoms than VPA-treated Met allele patients, likely due to the impact of VPA on proline level. Our data have implications for treatment decisions, because proline-modulating medications, such as VPA which is commonly used to treat schizophrenia patients, may have beneficial, and conversely detrimental effects on negative symptoms, based upon the individual patient’s Val158Met genotype.

In a second sample; inpatients with bipolar disorder, we explored the interaction between COMT and proline on negative-symptom change (using the BPRS-negative-symptom subscale). Supporting our earlier schizophrenia finding, we observed a significant interaction between proline and COMT: high proline was associated with improvement of negative symptoms in homozygous Val/Val bipolar disorder patients, whereas high proline in Met allele carriers was associated with less improvement or an increase in negative-symptom severity. This finding was not confounded by medication use, the duration of time between assessments, or demographic characteristics of the bipolar sample. Interestingly, the bipolar patients did not have proline levels significantly higher than controls, suggesting that in contrast with schizophrenia, proline may impact negative symptoms and their severity, but not bipolar disorder risk.

To our knowledge, this is the first study to document that proline and COMT interact to predict negative-symptom outcomes in psychiatric disorders. Our findings of a detrimental effect of high proline in combination with the COMT Met allele, on schizophrenia and bipolar disorder negative symptoms, is in part supported by studies of 22q11DS patients, who have an increased risk of psychosis (albeit exhibiting positive symptoms) plus a neurophysiological visual sensory deficit, when carrying the Met allele in the presence of high proline. Moreover, Hidding et al. recently reported the negative effect of high proline and hemizygous COMT Met on autism spectrum symptoms in children and adolescents with 22q11DS, which is particularly relevant given the notable symptom similarities between autism and schizophrenia spectrum disorders.

Our finding that high proline is protective in Val/Val patients with schizophrenia and bipolar disorder is also novel and significant. Intriguingly, Zarchi et al. had reported the protective effect of a PRODH variant (the Tryptophan (Trp) allele of the Arg185Trp polymorphism) on a neurophysiological measure; mismatch negativity in COMT Val 22q11DS patients. As the Trp allele exhibits decreased POX activity in vitro, Zarchi et al., discussed either an opposite effect of this allele in vivo, or alternatively that the Arg185Trp polymorphism is in linkage disequilibrium with another functional SNP; in each circumstance likely resulting in increased POX activity and low peripheral proline. Our current study data suggest the opposite to that interpretation: That high proline is actually protective in hemizygous 22q11DS patients with the Val genotype, with regards to mismatch negativity.

Putative CNS roles of proline have been described both in terms of its potential as a neurotransmitter, as suggested by its uptake into and direct synthesis within synaptosomes and its release at the synapse after K+ induced depolarization, as well as a neuromodulator of neurotransmitter systems, as suggested by the presence of high-affinity proline transporters in glutamatergic neurons, and the enhancements of glutamatergic and prefrontal dopamine transmission in the presence of Prodh deficiency and elevated proline. Moreover, proline metabolism to Δ1-pyrroline-5-carboxylate (P5C) and then glutamate, produces ATP, reactive oxygen species (particularly superoxide radicals), and the conversion of NAD+ to NADH, initiating multiple downstream metabolic signaling pathways. Although the mechanism by

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**Table 4.** Results: The interaction between proline and COMT predicts negative-symptom change in bipolar disorder

| Dependent Variable = % Change in BPRS Negative Symptoms Scale, n = 43 |
|-------------------------|-----------------|-----------------|---------------|-------------|
| **Initial Model**        | **β coefficient** | **s.e.**        | **Test statistic** | **P-value** |
| Proline                 | 0.0011           | 0.0006          | 1.69            | 0.099       |
| COMT (Met allele vs Val/Val) | 0.3834         | 0.1862          | 2.06            | 0.046*      |
| Interaction (COMT × proline) | −0.0017        | 0.0007          | −2.42           | 0.040*      |
| **Full Model**          | **β coefficient** | **s.e.**        | **Test statistic** | **P-value** |
| Proline                 | 0.0012           | 0.0006          | 2.09            | 0.044*      |
| COMT (Met Allele vs Val/Val) | 0.4281         | 0.1650          | 2.60            | 0.014*      |
| Interaction (COMT × proline) | −0.0017        | 0.0007          | −2.42           | 0.022*      |
| Gender                  | 0.1960           | 0.0655          | 2.99            | 0.005*      |
| Ethnicity               |                 |                 |                 |             |
| Hispanic vs Caucasian   | 0.0186           | 0.0839          | 0.22            | 0.826       |
| African American vs Hispanic | −0.1528        | 0.1052          | −1.45           | 0.156       |
| Duration (days) between assessments | 0.0049       | 0.0070          | 0.69            | 0.492       |
| Neuroleptic type        |                 |                 |                 |             |
| Atypical neuroleptic vs none | −0.0802       | 0.0818          | −0.98           | 0.334       |
| Typical Neuroleptic vs none | −0.1401       | 0.2087          | −0.67           | 0.507       |
| Both vs none            | −0.1531          | 0.1184          | −1.29           | 0.206       |
| Benzodiazepines         | −0.0840          | 0.0714          | −1.18           | 0.249       |

**Final Model**

| **β coefficient** | **s.e.** | **Test statistic** | **P-value** |
|-------------------|----------|--------------------|-------------|
| Proline           | 0.0016   | 0.0006             | 2.55        | 0.015*      |
| COMT (Met Allele vs Val/Val) | 0.5029   | 0.1766             | 2.85        | 0.007*      |
| Interaction (COMT × proline) | −0.0021 | 0.0007             | −2.83       | 0.007*      |
| Gender            | 0.1856   | 0.0656             | 2.83        | 0.007*      |

Abbreviations: BPRS, Brief Psychiatric Rating Scale; COMT, catechol-O-methyltransferase. (b) (bipolar models using linear regression). P = 0.056. Wald test: canonical linear hypothesis that combined effect of covariates (Ethnicity, Duration, Neuroleptic Type and use of Benzodiazepines, bold) is zero. Wald test: covariate effect (Gender, italic) is zero.
which proline elevation may impact neurotransmission requires further investigation, it is apparent from studies demonstrating alterations in the cell redox state and oxidative stress in conditions of high proline (reviewed in Wyse et al.49, the Prodh-null model,7,8 as well as the human hyperprolinemias5 and pyrroline-5-carboxylate reductase deficiencies50,51 that elevated proline and/or abnormalities in proline metabolism and biosynthesis, can be detrimental to the CNS.

In schizophrenia and bipolar disorder, presence of the COMT Met allele may further accentuate proline toxicity. For example, in one putative model, enhanced dopamine-transmission in the PFC as a result of excess proline is exacerbated by low COMT activity and concomitant higher prefrontal dopamine availability, ultimately resulting in a frontal hyperdopaminergic state that mimics the Prodh-null mouse5 (and reviewed in Drew et al.).9 A hyperdopaminergic model influencing negative symptoms is somewhat counterintuitive, given that negative symptoms are generally considered to arise from deficient mesocortical dopaminergic stimulation. However, COMT is involved in maintaining PFC cognitive stability23,53 and in situations of high cortical dopamine concentrations and D1 receptor stimulation (likely present in Met/Met and to a lesser degree Val/Met psychiatric patients), enhanced cognitive stability of neuronal network activation has been theorized to result in a cognitive rigidity that may increase the likelihood of negative symptoms.23

Conversely, we found that proline elevation beneficially influences negative-symptom outcomes in Val/Val patients. In a COMT Val homozygous state, high enzymatic activity in the PFC would likely reduce prefrontal dopamine, limiting D1 receptor-mediated excitation.23,53 Speculatively, proline elevation may increase prefrontal dopamine signaling, through interference with glutamatergic pathways, reducing vulnerability to a prefrontal hypodopaminergic state in Val/Val patients. Although COMT genotype may also interact with proline-induced altered cellular redox and increased oxidative stress, we consider that a parsimonious interpretation of currently available information regarding neuromodulatory effects of proline6 suggests that in the presence of elevated proline, negative symptoms are significantly impacted in conditions of both hyper- or hypodopaminergia, which is consistent with the PFC functioning model theorized by Mattay et al., in which the optimal balance of dopamine signaling falls within a narrow range, following an inverted U-shaped curve.54

Interestingly, we found no relationship between COMT and proline on positive symptoms in schizophrenia. Positive symptoms are considered to arise from hyperactive subcortical mesolimbic projections, and our finding is consistent with the action of proline in murine cortical but not striatal dopamine potentiation.8 In addition, dopamine transporters are relatively sparse in the PFC,55 and the removal of dopamine there may be more impacted by COMT activity and the interaction with proline, as compared with subcortical regions.

Some study limitations exist: in the schizophrenia sample, proline was measured and symptoms assessed cross-sectionally. Thus, our findings may be confounded by enrollment differences across genotypes, and one particularly relevant variable to consider regarding this limitation is the duration of illness and how the structure of positive and negative symptoms may change during the course of the illness. In our study, although the duration of illness was higher in Met/Met patients, negative symptoms were not significantly different between genotypes, there was no significant main effect of COMT on negative symptoms, and the length of current hospitalization prior to symptom assessment had no relationship with negative symptoms. Furthermore, the significant proline × COMT interaction remained following analysis performed in a bisected sample of short and long illness durations. Taken together, we consider that the cross-sectional nature of the study did not significantly confound our findings. In addition, although the bipolar study allowed initial investigation of symptom change, the bipolar sample size was smaller and negative symptoms were assessed using a subscale of the BPRS. Further research would therefore benefit from a longitudinal approach, investigating the interaction between proline and COMT on the change in negative symptoms assessed via the SANS or Positive and Negative Symptom Scale, in a large sample of both schizophrenia and bipolar disorder patients.

Nonetheless, there are currently no medications approved for the treatment of negative symptoms in psychiatric illness, which are associated with poor functional outcomes and quality of life, are highly persistent, and are a great burden for caregivers.2 Our finding of a beneficial effect on negative symptoms of high proline in Val/Val inpatients suggests that personalization of treatments based upon a patient’s COMT genotype, for the purpose of up- or downregulating proline level should be further investigated, as it may hold promise as a pharmacogenomics approach to intervene and target this symptom domain.

CONFLICT OF INTEREST

This research was supported by grants from the National Institutes of Health (NIH, as follows: R21MH0706019 (JC), R21MH082331 (JC) and R01MH100219 (CC) from the National Institute of Mental Health. In addition, this research was supported in part by New York University CTSA grant (UL1 TR000038) and Columbia University Medical Center CTSA grant (KL2 RR024157: KL2 awardee CC), from the National Center for Advancing Translational Sciences. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. CLC and JDC are inventors on two patent applications that are based in part upon this study data. If awarded, the patents will be owned by their respective institutions, and CLC and JDC may benefit financially in the future if these patents are licensed. CLC and JDC declare no other financial relationships that are directly or indirectly related to this work. The remaining authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank Ellie DeCandia RN and the nursing staff of the New York University CTSI, for their invaluable assistance during this study.

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COMT, proline and negative psychiatric symptoms

CL Clelland et al

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