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Positive Symptoms of Psychosis Correlate With Expression of Ubiquitin Proteasome Genes in Peripheral Blood

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Abstract

Several brain- and blood-based gene expression studies in patients with psychotic disorders (e.g., schizophrenia) have identified genes in the ubiquitin proteasome system (UPS) pathway as putative biomarkers. However, to date an examination of the UPS pathway in the broader context of symptom severity in psychosis has not been conducted. The purpose of this study was to investigate the correlation between clinical scores on the Scales for the Assessment of Positive and Negative Symptoms (SAPS–SANS) and expression of 43 highly annotated genes within the UPS pathway in blood from patients with psychosis. A sample of 19 psychotic patients diagnosed with schizophrenia (n = 13) or bipolar disorder (n = 6) were recruited. Pearson's partial correlations, adjusting for gender, ethnicity, age, education, medication, smoking, and past 6-month substance use, were performed between each of the selected UPS genes and both scales. Significant Bonferroni-adjusted positive associations were observed between SAPS scores and two ubiquitin conjugation genes (i.e., *UBE2K*, *SIAH2*), while a negative association was observed with one deubiquitination gene (i.e., *USP2*). No gene expression levels were significantly associated with scores on the SANS after correction for multiple testing. Our findings suggest that dysregulation of the UPS, specifically ubiquitin conjugation and deubiquitination, may point to a possible underlying biological mechanism for severity of positive but not negative symptoms.

Keywords

UBE2K; E2-25K/Hip-2; SIAH2; USP2; UBP41

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The ubiquitin proteasome system (UPS) is a highly complex, temporally controlled, and tightly regulated process that plays major roles in a variety of basic cellular processes, specifically degradation of intracellular proteins [Ciechanover et al., 2000]. The UPS has been implicated in a variety of human diseases including several neurodegenerative diseases [Ciechanover and Brundin, 2003] such as Parkinson disease [Shimura et al., 2001] and Alzheimer disease [Lam et al., 2000]. Furthermore, several studies of psychosis using post-mortem brain tissue have provided evidence of dysregulated expression of several UPS-related genes [Vawter et al., 2001, 2002; Middleton et al., 2002; Konradi et al., 2004; Altar et al., 2005]. Among these studies, three [Vawter et al., 2001; Middleton et al., 2002; Altar et al., 2005] reported ubiquitin carboxyl-terminal esterase L1 (*UCHL1*) as significantly down-regulated and two showed several proteasome 26S subunits as significantly down-regulated in schizophrenia [Vawter et al., 2001; Altar et al., 2005]. These studies also showed significant down-regulation of ubiquitin B (*UBB*) [Altar et al., 2005], ubiquitin-specific peptidase 9 (*USP9*) [Vawter et al., 2001], ubiquitin-specific peptidase 14 (*USP14*) [Middleton et al., 2002], and ubiquitin conjugating enzyme E2N (*UBE2N*) [Vawter et al., 2002] in schizophrenia. In addition, using a pathway-analytic approach, we have demonstrated in two independent samples a dysregulation of the UPS in psychosis [Bousman et al., 2009]. However, to date nothing is known about the relationship between the UPS and symptom severity in psychotic disorders. Thus, based on previous gene expression data collected by our group and others, we sought to correlate blood-based expression of genes within the UPS with symptom severity indices of patients with a history of psychosis (i.e., schizophrenia or bipolar disorder). Specifically, we sought to estimate the association of each gene's expression with clinically assessed positive and negative symptoms.

Clinical and gene expression data from 19 subjects (Table I) meeting DSM-IV criteria for at least one episode of psychosis were acquired from a larger gene expression study of blood-based biomarkers of schizophrenia and bipolar disorder (GEO Accession number: GSE18312). Subjects were excluded if any of the following were present: (1) substance abuse or dependence in the past year; (2) neurologic problems (e.g., stroke, meningitis); (3) systemic medical illnesses (e.g., heart disease, diabetes); (4) history of head injury with documented loss of consciousness lasting longer than 10 min; (5) pregnancy; or (6) physical disabilities. More details on the recruitment, inclusion, and exclusion criteria as well as procedures used to diagnose psychosis were described previously [Bousman et al., 2009]. Whole blood samples (10 ml) were collected into EDTA-coated collection tubes in the morning after subjects fasted overnight. Samples were then transferred immediately to an RNase-free laboratory, where all subsequent procedures took place. Collection and preparation of blood samples, separation and lysis of PBMCs, extraction, purification, and hybridization of RNA, quantification of expression levels on GeneChip® Human Exon 1.0 ST Arrays (Affymetrix, Inc.; Santa Clara, CA), and quality-control procedures were all performed by standard methods, which are described in greater detail elsewhere [Bousman et al., 2009; Glatt et al., 2009]. All study procedures were approved by the Institutional Review Board at University of California, San Diego.

As part of the clinical characterization procedures, the Scales for the Assessment of Positive and Negative Symptoms (SAPS–SANS) [Andreasen and Olsen, 1982] were administered to all subjects by a trained Master's-level research assistant and later reviewed by two independent doctoral-level clinicians to ensure accurate scoring. The SAPS assesses four distinct positive symptom subcategories (i.e., hallucinations, delusions, bizarre behavior, and thought disorder), whereas the SANS assesses five negative symptom subcategories (i.e., affective flattening, avolition, anhedonia-asociality, and attention) on a severity scale of 0 (none) to 5 (severe). For the current study, the four and five subcategory scores were averaged to create global scores for positive (SAPS mean = 1.6, SD = 0.6) and negative (SANS mean = 1.5, SD = 0.8) symptoms, respectively (Table I). Although some subjects reported no negative symptoms at assessment, all subjects reported mild (score = 2) or greater severity on one or more of the positive symptom subscales.

The number of genes which are thought to populate the UPS is continually changing and a consensus on the number has not been achieved. According to one of the largest knowledge bases of biological networks (Ingenuity Pathway Analysis, version 7.6; Ingenuity Systems®, Redwood City, CA), the UPS contains 201 genes. In an effort to include as many of these genes as possible in the current analysis, Affymetrix's probeset IDs for each of these genes were acquired and cross-referenced with the “core” expression data for the Exon 1.0 ST Array. We chose to use the “core” versus “extended” or “full” expression data due to the former's inclusion of highly annotated genes and exclusion of hypothetical gene transcripts without clear biological relevance. This process resulted in the selection of 43 highly annotated UPS pathway genes. Although it is a possibility that many genes that were not included would provide further insight into the UPS pathway as it relates to psychotic symptoms, it is possible that the 43 selected genes serve as an adequate proxy of the UPS pathway. In fact, the 43 selected genes represented the six major processes in the UPS pathway: (1) ubiquitin encoding (n = 2 genes), (2) ubiquitin activation (n = 2 genes), (3) ubiquitin conjugation (n = 21 genes), (4) protein degradation (n = 7 genes), or (5) deubiquitination (n = 11 genes).

Raw cell intensity (.CEL) files were generated from gene chip image files using GCOS version 1.0 (Affymetrix). Resulting .CEL files were imported into Partek Genomics Suite (Partek Inc, St. Louis, MO) using robust multichip average (RMA) to normalize gene expression intensities. Partek was then used to correlate each of the 43 selected UPS genes' intensities with SAPS–SANS global severity scores using Pearson partial correlations, adjusting for gender, ethnicity, age, education, medication (i.e., antipsychotics and/or mood stabilizers), current smoking (yes/no), and past 6-month substance use (yes/no). Due to the large number of statistical tests performed, the probability of committing type I errors (i.e., finding false-positive results) in this study was inflated. This threat was addressed statistically using a Bonferroni correction. Correlations between the 43 genes for both the SAPS and SANS summed to a total of 86 hypothesis tests, resulting in an adjusted alpha threshold of $0.05/86 = 5.81 \times 10^{-4}$.

Three (*UBE2K*, *SIAH2*, *USP2*) of the 43 UPS genes examined were significantly correlated with scores on the SAPS, after *P*-values were adjusted for multiple testing. *UBE2K* and *SIAH2* showed positive correlations ($r = 0.74$, $P = 0.0005$ and $r = 0.73$, $P = 0.0005$;

respectively), whereas *USP2* was negatively correlated with the SAPS ($r = -0.76$, $P = 0.0005$; Table II; Supplemental Figure). Post hoc exploration of these three significant genes for each of the four subscales of the SAPS revealed significant positive correlations between hallucination severity and *UBE2K* ($r = 0.67$, $P = 0.002$) and *SIAH2* ($r = 0.64$, $P = 0.003$) expression. None of the SAPS subscales were significantly correlated with *USP2* expression after multiple-testing correction (α : $0.05/12 = 0.004$), although delusion severity and *USP2* expression were negatively correlated at a nominal level of significance ($r = -0.67$, $P = 0.005$).

None of the 43 gene transcripts examined were shown to correlate with severity scores on the SANS, although *UBE2K* had a nominally significant positive correlation with the SANS severity score ($r = 0.60$, $P = 0.007$).

We were unable to perform QRTPCR validation for the three significantly correlated genes in our sample due to inadequate mRNA quantities; although, previous work with the current sample has successfully validated genes using QRTPCR [Bousman et al., 2009]. Thus, our results provide preliminary evidence that UPS gene expression in peripheral blood mononuclear cells is correlated with positive (i.e., hallucinations) but not negative symptoms in psychosis. Two ubiquitin conjugating (i.e., *UBE2K*, *SIAH2*) genes and one deubiquitinating (i.e., *USP2*) gene were significantly correlated with the positive symptom severity score (i.e., SAPS). To our knowledge, none of these genes has been identified in previous gene expression studies in psychosis or other major psychiatric disorders as putative biomarkers. However, several related genomic and neurobiological studies in psychosis (as well as neurodegenerative diseases) can be used to assist in interpreting our findings.

Ubiquitination by *UBE2K*, an E2 ubiquitin conjugating enzyme, enhances the aggregate formation of expanded polyglutamine proteins and the suppression of apoptosis in polyglutamine diseases (e.g., Huntington disease) [de Pril et al., 2007] as well as inhibits proteasome function in vitro [Song et al., 2003]. Previous research has shown expansion of polyglutamine tracts in schizophrenia [Joobert et al., 1999; Denghien et al., 2000] and more recently, these tracts were shown to be inversely correlated with age of onset and prefrontal cortical functioning in schizophrenia [Ohi et al., 2009]. This previous work suggests that our finding of up-regulation in *UBE2K* is associated with greater severity of positive symptoms and is potentially mediated by increased aggregate formation of polyglutamine tracts and/or proteasome inhibition. Future in vivo, in vitro, and postmortem investigations will need to verify this potential mechanism and its contribution to positive symptom severity in psychosis.

The majority of research to date involving *SIAH2*, an E3 ubiquitin conjugating enzyme, has been focused on its link with hypoxia [Nakayama et al., 2009]. However, recent work has reported that *SIAH2* directly interacts with and mono-ubiquitinates alpha-synuclein, promoting its aggregation in vitro and in vivo, resulting in toxicity in dopaminergic cells [Rott et al., 2008]. Reductions in alpha-synuclein have been reported in bipolar disorder [Scarr et al., 2006] and polymorphisms within the alpha-synuclein gene have been associated with methamphetamine-psychosis [Kobayashi et al., 2004]. In addition,

amplification of alpha-synuclein has been associated with pronounced visual, auditory, and olfactory hallucinations among subjects with Parkinson disease [Fuchs et al., 2007; Ross et al., 2008]. Thus, it is possible that the correlation between up-regulation of *SIAH2* and greater positive symptom severity, in the current study, is in part being driven via alpha-synuclein aggregation and/or dopaminergic neurotoxicity. However, the mechanistic reason for aggregation and neurotoxicity subsequent to mono-ubiquitination by *SIAH2* is still unclear and warrants further investigation.

Thus far, focus has been given to ubiquitin conjugation or tagging of proteins for degradation as a potential correlate of positive symptom severity in psychosis. However, an equally important step in the UPS pathway is deubiquitination. Deubiquitination enzymes (DUBs) such as *USP2*, are involved in multiple functions that remove intact ubiquitin from target proteins (i.e., rescue the protein from degradation) and/or impact levels of free intracellular ubiquitin pools required for ubiquitin-dependent functions in the cell [D'Andrea and Pellman, 1998; Tirat et al., 2005]. DUBs have been the least studied enzymes of the UPS; however, previous work in schizophrenia has demonstrated down-regulation of *UCHL1* [Vawter et al., 2001; Middleton et al., 2002; Altar et al., 2005], *USP9* [Vawter et al., 2001], and *USP14* [Middleton et al., 2002], three of 80–90 DUBs found in mammals [Singhal et al., 2008]. Similar to *USP9* and *USP14*, *USP2* is a cysteine peptidase; that preferentially cleaves ubiquitin from larger molecules, such as proteins [Gewies and Grimm, 2003]. Gewies and Grimm [2003] showed *USP2* overexpression in 293T and HeLa human cell lines, resulted in dramatic deubiquitination of a broad range of proteins and directly induced apoptosis. In the current study, we observed an inverse correlation between *USP2* expression and positive symptom severity suggesting a potential clinical correlate of deubiquitination of *USP2* substrates and apoptosis. However, our finding appears to be in conflict with that of Gewies et al. [2003] in that we would expect that deubiquitination and apoptosis would be detrimental to UPS functioning and subsequently correlate with greater severity in positive symptoms. Clearly, additional research is required to elucidate further the role of *USP2* as well as other DUBs in the manifestation of positive symptom severity in psychosis.

Correlations with positive and/or negative symptom severity among genes classified as ubiquitin encoding, ubiquitin activating, or 26S proteasome subunits were not detected. However, one study has reported down-regulation of *UBB*, an ubiquitin encoding gene, and two studies [Vawter et al., 2001; Altar et al., 2005] have identified several regulatory subunits in the 26S proteasome, as significantly down-regulated in schizophrenia. Thus, ubiquitin encoding and/or the 26S proteasome, which is responsible for degradation of most ubiquitinated proteins, may also harbor gene transcripts involved in the pathogenesis of psychosis and/or symptom severity. Our ability to uncover significant gene correlates involved in the UPS, particularly in the 26S proteasome and ubiquitin encoding, may have been inhibited by several limitations within our study. First, the sample was small and may have prohibited us from detecting correlations that would have attained statistical significance in a larger sample. In fact, examination of large expression effects ($r > \pm 0.49$) [Cohen, 1992] for positive and/or negative symptoms suggest one gene involved in ubiquitin activation (*SAE1*), six involved in ubiquitin conjugation (*UBE2D2*, *CHFR*, *SIAH2*, *UBE3C*,

UBOX5, *UBR2*), two involved in the 26S proteasome (*PSMC3*, *PSMD1*), and two involved in deubiquitination (*USP5*, *USP21*) may have been significant in a larger sample. Second, we included subjects with schizophrenia or bipolar disorder with a history of psychosis. This decision was made on the assumption that psychosis in both disorders operates under similar biological mechanisms and that an increase in the sample size and subsequent power would outweigh any potential statistical noise introduced by combining the two disorders. Third, we adjusted for current psychotropic medications, a standard analytical practice, yet type and dose of medication(s) were unknown and could have artificially reduced our observed correlations with symptom severity. However, post hoc analysis removing adjustments for medication, showed no difference in our reported results. Finally, we utilized a blood-based gene expression approach and it remains unclear whether molecular signatures in blood accurately reflect those found in the brain. However, several investigators have reported that the circulating blood may act as a “sentinel tissue” [Liew, 1999], “neural probe” [Gladkevich et al., 2004], or “surrogate” [Sullivan et al., 2006] for underlying pathophysiology in psychiatric disorders. Nevertheless, post-mortem expression studies with access to ante-mortem clinical data would further assist in elucidating the role of the UPS in psychosis symptom severity. Future research examining expression of a larger pool of UPS genes using more sensitive techniques (e.g., QRT-PCR) as well as studies exploring UPS protein levels and in vitro the effects of antipsychotic medications on UPS gene expression are also warranted. Longitudinal studies designed to examine gene expression prior to, during, and after a psychotic episode would also greatly elucidate the role of the UPS in psychosis severity and aid in identification of potential therapeutic targets.

In conclusion, our results support previous work and provide preliminary data suggesting dysregulation of ubiquitin conjugation and deubiquitination being one, of potentially many, biological mechanisms mediating the manifestation and severity of positive psychotic symptoms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

- Altar CA, Jurata LW, Charles V, Lemire A, Liu P, Bukhman Y, Young TA, Bullard J, Yokoe H, Webster MJ, Knable MB, Brockman JA. Deficient hippocampal neuron expression of proteasome, ubiquitin, and mitochondrial genes in multiple schizophrenia cohorts. *Biol Psychiatry*. 2005; 58:85–96. [PubMed: 16038679]
- Andreassen NC, Olsen S. Negative/positive schizophrenia. Definition and validation. *Arch Gen Psychiatry*. 1982; 39:789–794. [PubMed: 7165478]

- Bousman CA, Chana G, Glatt SJ, Chandler SD, Lucero GR, Tatro E, May T, Lohr JB, Kremen WS, Tsuang MT, Everall IP. Preliminary evidence of ubiquitin proteasome system dysregulation in schizophrenia and bipolar disorder: Convergent pathway analysis findings from two independent samples. *Am J Med Genet Part B*. 2009; 153B:494–502. [PubMed: 19582768]
- Ciechanover A, Brundin P. The ubiquitin proteasome system in neurodegenerative diseases: Sometimes the chicken, sometimes the egg. *Neuron*. 2003; 40:427–446. [PubMed: 14556719]
- Ciechanover A, Orian A, Schwartz AL. Ubiquitin-mediated proteolysis: Biological regulation via destruction. *Bioessays*. 2000; 22:442–451. [PubMed: 10797484]
- Cohen J. A power primer. *Psychol Bull*. 1992; 112:155–159. [PubMed: 19565683]
- D'Andrea A, Pellman D. Deubiquitinating enzymes: A new class of biological regulators. *Crit Rev Biochem Mol Biol*. 1998; 33:337–352. [PubMed: 9827704]
- de Pril R, Fischer DF, Roos RA, van Leeuwen FW. Ubiquitin-conjugating enzyme E2-25K increases aggregate formation and cell death in polyglutamine diseases. *Mol Cell Neurosci*. 2007; 34:10–19. [PubMed: 17092742]
- Denghien I, Joobar R, Rouleau GA, Neri C. Polyglutamine tracts in schizophrenia: Gaining new insights. *Mol Psychiatry*. 2000; 5:236–237. [PubMed: 10889523]
- Fuchs J, Nilsson C, Kachergus J, Munz M, Larsson EM, Schule B, Langston JW, Middleton FA, Ross OA, Hulihan M, Gasser T, Farrer MJ. Phenotypic variation in a large Swedish pedigree due to SNCA duplication and triplication. *Neurology*. 2007; 68:916–922. [PubMed: 17251522]
- Gewies A, Grimm S. UBP41 is a proapoptotic ubiquitin-specific protease. *Cancer Res*. 2003; 63:682–688. [PubMed: 12566314]
- Gladkevich A, Kauffman HF, Korf J. Lymphocytes as a neural probe: Potential for studying psychiatric disorders. *Prog Neuropsychopharmacol Biol Psychiatry*. 2004; 28:559–576. [PubMed: 15093964]
- Glatt SJ, Chandler SD, Bousman CA, Chana G, Lucero GR, Tatro E, May T, Lohr JB, Kremen WS, Everall IP, Tsuang MT. Alternatively spliced genes as biomarkers for schizophrenia, bipolar disorder and psychosis: A blood-based spliceome-profiling exploratory study. *Curr Pharmacogenomics Personalized Med*. 2009; 7:164.
- Joobar R, Benkelfat C, Jannatipour M, Turecki G, Lal S, Mandel JL, Bloom D, Lalonde P, Lopes-Cendes I, Fortin D, Rouleau G. Polyglutamine-containing proteins in schizophrenia. *Mol Psychiatry*. 1999; 4:53–57. [PubMed: 10089009]
- Kobayashi H, Ide S, Hasegawa J, Ujike H, Sekine Y, Ozaki N, Inada T, Harano M, Komiyama T, Yamada M, Iyo M, Shen HW, Ikeda K, Sora I. Study of association between alpha-synuclein gene polymorphism and methamphetamine psychosis/dependence. *Ann NY Acad Sci*. 2004; 1025:325–334. [PubMed: 15542733]
- Konradi C, Eaton M, MacDonald ML, Walsh J, Benes FM, Heckers S. Molecular evidence for mitochondrial dysfunction in bipolar disorder. *Arch Gen Psychiatry*. 2004; 61:300–308. [PubMed: 14993118]
- Lam YA, Pickart CM, Alban A, Landon M, Jamieson C, Ramage R, Mayer RJ, Layfield R. Inhibition of the ubiquitin-proteasome system in Alzheimer's disease. *Proc Natl Acad Sci USA*. 2000; 97:9902–9906. [PubMed: 10944193]
- Liew CC, Ma J, Tang HC, Zheng R, Dempsey AA. The peripheral blood transcriptome dynamically reflects system wide biology: A potential diagnostic tool. *J Lab Clin Med*. 2006; 147:126–132. [PubMed: 16503242]
- Middleton FA, Mirnics K, Pierri JN, Lewis DA, Levitt P. Gene expression profiling reveals alterations of specific metabolic pathways in schizophrenia. *J Neurosci*. 2002; 22:2718–2729. [PubMed: 11923437]
- Nakayama K, Qi J, Ronai Z. The ubiquitin ligase Siah2 and the hypoxia response. *Mol Cancer Res*. 2009; 7:443–451. [PubMed: 19372575]
- Ohi K, Hashimoto R, Yasuda Y, Kiribayashi M, Iike N, Yoshida T, Azechi M, Ikezawa K, Takahashi H, Morihara T, Ishii R, Tagami S, Iwase M, Okochi M, Kamino K, Kazui H, Tanaka T, Kudo T, Takeda M. TATA box-binding protein gene is associated with risk for schizophrenia, age at onset and prefrontal function. *Genes Brain Behav*. 2009; 8:473–480. [PubMed: 19566714]

- Ross OA, Braithwaite AT, Skipper LM, Kachergus J, Hulihan MM, Middleton FA, Nishioka K, Fuchs J, Gasser T, Maraganore DM, Adler CH, Larvor L, Chartier-Harlin MC, Nilsson C, Langston JW, Gwinn K, Hattori N, Farrer MJ. Genomic investigation of alpha-synuclein multiplication and Parkinsonism. *Ann Neurol*. 2008; 63:743–750. [PubMed: 18571778]
- Rott R, Szargel R, Haskin J, Shani V, Shainskaya A, Manov I, Liani E, Avraham E, Engelender S. Monoubiquitylation of alpha-synuclein by seven in absentia homolog (SIAH) promotes its aggregation in dopaminergic cells. *J Biol Chem*. 2008; 283:3316–3328. [PubMed: 18070888]
- Scarr E, Gray L, Keriakous D, Robinson PJ, Dean B. Increased levels of SNAP-25 and synaptophysin in the dorsolateral prefrontal cortex in bipolar I disorder. *Bipolar Disord*. 2006; 8:133–143. [PubMed: 16542183]
- Shimura H, Schlossmacher MG, Hattori N, Frosch MP, Trockenbacher A, Schneider R, Mizuno Y, Kosik KS, Selkoe DJ. Ubiquitination of a new form of alpha-synuclein by Parkin from human brain: Implications for Parkinson's disease. *Science*. 2001; 293:263–269. [PubMed: 11431533]
- Singhal S, Taylor MC, Baker RT. Deubiquitylating enzymes and disease. *BMC Biochem*. 2008; 9(Suppl1):S3. [PubMed: 19007433]
- Song S, Kim SY, Hong YM, Jo DG, Lee JY, Shim SM, Chung CW, Seo SJ, Yoo YJ, Koh JY, Lee MC, Yates AJ, Ichijo H, Jung YK. Essential role of E2-25K/Hip-2 in mediating amyloid-beta neurotoxicity. *Mol Cell*. 2003; 12:553–563. [PubMed: 14527403]
- Sullivan PF, Fan C, Perou CM. Evaluating the comparability of gene expression in blood and brain. *Am J Med Genet Part B*. 2006; 141B:261–268. [PubMed: 16526044]
- Tirat A, Schilb A, Riou V, Leder L, Gerhartz B, Zimmermann J, Worpenberg S, Eidhoff U, Freuler F, Stettler T, Mayr L, Ottl J, Leuenberger B, Filipuzzi I. Synthesis and characterization of fluorescent ubiquitin derivatives as highly sensitive substrates for the deubiquitinating enzymes UCH-L3 and USP-2. *Anal Biochem*. 2005; 343:244–255. [PubMed: 15963938]
- Vawter MP, Barrett T, Cheadle C, Sokolov BP, Wood WH, Donovan DM, Webster M, Freed WJ, Becker KG. Application of cDNA microarrays to examine gene expression differences in schizophrenia. *Brain Res Bull*. 2001; 55:641–650. [PubMed: 11576761]
- Vawter MP, Crook JM, Hyde TM, Kleinman JE, Weinberger DR, Becker KG, Freed WJ. Microarray analysis of gene expression in the prefrontal cortex in schizophrenia: A preliminary study. *Schizophr Res*. 2002; 58:11–20. [PubMed: 12363385]

TABLE I

Sample Characteristics

Variable	Total (n = 19)
Age: mean, years (SD)	43 (9)
Sex: female, n (%)	6 (32)
Education: mean, years (SD)	13 (2)
Ancestry: n (%)	
European	10 (53)
African	6 (32)
Hispanic	2 (10)
Asian	1 (5)
Current smoker: n (% yes)	13 (68)
Past 6-month substance use: n (% yes)	3 (16)
Current medication use: n (% yes)	
Antipsychotic	16 (84)
Mood stabilizer	7 (37)
History of psychosis: n (% yes)	19 (100)
Positive symptoms: mean score (SD) ^a	
Hallucinations	2.8 (1.7)
Delusions	2.7 (1.3)
Bizarre behaviors	0.3 (0.5)
Formal thought disorder	0.5 (0.8)
Positive symptom total (SAPS)	1.6 (0.6)
Negative symptoms: mean score (SD) ^a	
Affective flattening	1.9 (1.4)
Alogia	1.2 (1.5)
Apathy	2.2 (1.5)
Anhedonia	2.0 (1.7)
Attention	0.3 (0.6)
Negative symptom total (SANS)	1.5 (0.8)

SAPS, scale for the assessment of positive symptoms; SANS, scale for the assessment of negative symptoms.

^a Higher scores reflect greater severity (range: 0–5).

TABLE II

Ubiquitin Proteasome System Correlates of Positive and Negative Symptoms

Category	gene symbol	Gene name	Pearson's r (adjusted)			
			SAPS	P-value	SANS	P-value
Ub encoding						
	UBB	Ubiquitin B	0.29	ns	0.11	ns
	UBC	Ubiquitin C	0.32	ns	−0.05	ns
Ub activation (E1)						
	SAE1	SUMO1 activating enzyme subunit 1	0.59	0.008	−0.06	ns
	UBA1	Ubiquitin-like modifier activating enzyme 1	0.05	ns	0.02	ns
Ub conjugation (E2)						
	UBE2D1	Ubiquitin-conjugating enzyme E2D 1	−0.11	ns	0.22	ns
	UBE2D2	Ubiquitin-conjugating enzyme E2D 2	0.54	0.016	0.40	ns
	UBE2D3	Ubiquitin-conjugating enzyme E2D 3	0.32	ns	0.31	ns
	UBE2K	Ubiquitin-conjugating enzyme E2K	0.74	0.0005	0.60	0.007
Ub Conjugation (E3)						
	ANAPC11	Anaphase promoting complex subunit 11	0.29	ns	−0.13	ns
	CHFR	Checkpoint with forkhead and ring finger domains	−0.59	0.008	−0.31	ns
	CUL1	Cullin 1	0.39	ns	0.38	ns
	CUL2	Cullin 2	0.45	ns	0.28	ns
	CUL5	Cullin 5	−0.21	ns	−0.21	ns
	ITCH	Itchy E3 ubiquitin protein ligase homolog	0.13	ns	0.25	ns
	MDM2	Mdm2 p53 binding protein homolog	0.18	ns	0.13	ns
	NEDD4	Neural precursor cell expressed, developmentally down-regulated 4	0.24	ns	0.08	ns
	PARK2	Parkinson disease 2, Parkin	−0.24	ns	−0.34	ns
	SIAH2	Seven in absentia homolog 2	0.73	0.0005	0.46	0.047
	SMURF1	SMAD-specific E3 ubiquitin protein ligase 1	0.25	ns	0.20	ns
	UBE3A	Ubiquitin protein ligase E3A	−0.22	ns	0.05	ns
	UBE3C	Ubiquitin protein ligase E3C	−0.60	0.007	−0.24	ns
	UBOX5	U-box domain containing 5	−0.63	0.004	−0.51	0.025
	UBR2	Ubiquitin protein ligase E3 n-recognin 2	0.54	0.018	0.28	ns
	UBR5	Ubiquitin protein ligase E3 n-recognin 5	0.16	ns	0.15	ns
	WWP1	WW domain containing E3 ubiquitin protein ligase 1	0.47	0.042	0.41	ns
Degradation						
	PSMC2	Proteasome 26S subunit, ATPase, 2	0.34	ns	0.09	ns
	PSMC3	Proteasome 26S subunit, ATPase, 3	−0.19	ns	−0.50	0.030
	PSMC4	Proteasome 26S subunit, ATPase, 4	0.35	ns	0.01	ns
	PSMC6	Proteasome 26S subunit, ATPase, 6	−0.36	ns	−0.11	ns
	PSMD1	proteasome 26S subunit, non-ATPase, 1	−0.52	0.022	−0.44	ns
	PSMD13	Proteasome 26S subunit, non-ATPase, 13	0.13	ns	0.48	0.036
	PSMD14	Proteasome 26S subunit, non-ATPase, 14	0.48	0.039	0.09	ns
Deubiquitination						

Category	gene symbol	Gene name	Pearson's r (adjusted)			
			SAPS	P-value	SANS	P-value
	UCHL1	Ubiquitin carboxyl-terminal esterase L1	-0.26	ns	-0.18	ns
	UCHL3	Ubiquitin carboxyl-terminal esterase L3	0.35	ns	0.40	ns
	USP2	Ubiquitin specific peptidase 2	-0.76	0.0005	-0.35	ns
	USP5	ubiquitin specific peptidase 5	-0.20	ns	-0.37	ns
	USP7	Ubiquitin-specific peptidase 7	-0.46	0.048	-0.35	ns
	USP10	Ubiquitin-specific peptidase 10	0.38	ns	0.07	ns
	USP13	Ubiquitin-specific peptidase 13	-0.11	ns	-0.28	ns
	USP14	ubiquitin specific peptidase 14	-0.03	ns	-0.09	ns
	USP21	Ubiquitin-specific peptidase 21	0.52	0.023	0.27	ns
	USP22	Ubiquitin-specific peptidase 22	-0.35	ns	-0.10	ns
	USP33	Ubiquitin-specific peptidase 33	0.04	ns	-0.16	ns

Bold values are significant at a Bonferroni $P = 5.81 \times 10^{-4}$.