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Differing Levels of Ubiquitinated Proteins and Gene Expression in the Prefrontal Cortex in Schizophrenia and Bipolar Disorder

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Background

The etiology and pathophysiology of schizophrenia (SCZ) and bipolar disorder (BD) are not yet understood. The ubiquitin-proteasome system (UPS) is the principal mechanism for targeted degradation of cellular proteins and influences a diverse array of cellular processes, including regulation of gene transcription, the cell cycle, endocytosis, DNA repair and inflammatory response. Proteins are tagged for degradation with with single ubiquitin molecules or polyubiquitin chains. Polyubiquitylation on lysine K48 is associated with degradation by the proteasome, while polyubiquitylation on other lysines (e.g. K63) and monoubiquitylation regulates endocytic trafficking, inflammation, gene translation and DNA repair. Dysfunction of the UPS may link the many disparate abnormalities previously reported in SCZ and BD. To date, evidence for the involvement of the UPS in SCZ and BD comes primarily from transcriptomic studies[1-3], as such, additional targeted investigation at the protein level may help elucidate the role of the UPS in these disorders.

Aims and Objectives

In this study we quantified levels of free ubiquitin and K48 and K63 ubiquitylation in postmortem brain tissue from SCZ, BD and control subjects, as well as mRNA expression of the ubiquitin genes UBA, UBB, and UBC.

Methods

Samples: Postmortem brain tissue from prefrontal grey matter of control (n=35), SCZ (n=35) and BD (n=34) subjects was obtained from the Stanley Medical Research Institute Brain Bank (Table 1).

Protein quantification: Levels of free ubiquitin molecules and K48 and K63-linked ubiquitin chains were quantified by immunoblotting.

mRNA quantification: Expression of the ubiquitin genes UBA, UBB and UBC were quantified by qPCR.

Statistics: Between-group differences were investigated using ANCOVA, with diagnosis, sex, and diagnosis * sex interaction included in the models. Exploratory analyses were performed to investigate the influence of medications, body mass index (BMI), brain pH, post-mortem interval (PMI), and serum c-reactive protein (CRP) levels.

Table 1. Subjects and Demographics

	CON (n=35)	BD (n=34)	SCZ (n=35)
Age (years, mean, SD)	44.2 (7.6)	45.4 (10.7)	42.6 (8.5)
Sex (male/female)	26/9	16/18	26/9
PMI (hours, mean, SD)	29.4 (12.9)	37.9 (18.6)	31.4 (15.5)
Brain pH (mean, SD)	6.6 (0.3)	6.4 (0.3)	6.5 (0.2)
RIN (mean, SD)	7.2 (0.9)	7.3 (0.9)	7.4 (0.6)
Brain hemisphere (right:left)	16:19	19:15	17:18
Body mass index (mean, SD)	30.9 (9.0)	28.9 (10.1)	31.5 (8.2)
Age of onset (years, mean, SD)	n/a	25.3 (9.2)	21.3 (6.1)
Duration of illness (years, SD)	n/a	20.2 (9.5)	21.3 (10.2)
Antipsychotic dose (flu eq, mean, SD)	n/a	10212 (22871)	85004 (100335)
Mood stabilizer	0	23	11
Antidepressant	0	19	9
Alcohol history (none/social:moderate/heavy)	30:5	12:21	17:18
Smoking at death (no:yes)	9:9	6:15	4:23

Results

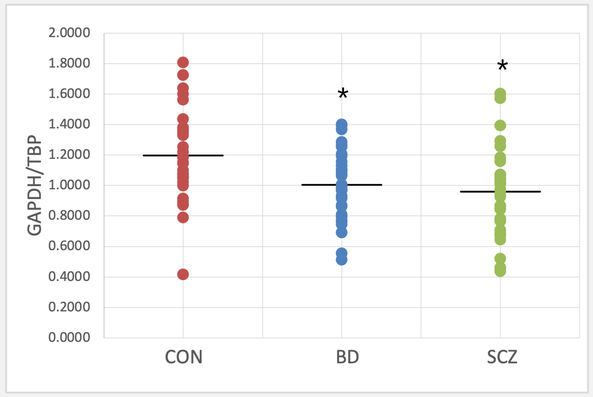
Ubiquitin Gene Levels:

Gene:	CON mean	BD mean	SCZ mean	Significant
UBC	1.198	1.004	0.959	significant
UBB	0.650	0.725	0.703	ns
UBA	0.561	0.640	0.596	ns

Table 2. *Mean gene expression of UBC, UBB, and UBA.*

There was no significant difference in gene expression in UBB or UBA between groups, however, UBC was lower in both SCZ and BD (Figure 1).

Figure 1. UBC Gene Expression.



Expression of ubiquitin gene UBC in control (CON), bipolar disorder (BD), and schizophrenia (SCZ). UBC gene expression differed between groups (p=0.002), being lower in BD (p=0.002) and SCZ (p=0.00008) compared to CON (p<0.05).*

Ubiquitin Protein Levels:

Protein:	CON	BD	SCZ	Significant
Ub-22kD	0.882	1.016	1.129	ns
Ub-9Kd	0.973	0.971	1.041	ns
K63- band 1	0.992	0.987	0.995	ns
K63- band 2	0.998	0.971	1.030	ns
K63- band 3	0.980	0.991	0.993	ns
K63- band 4	0.959	1.011	0.999	ns
K63- band 5	0.988	1.001	0.971	ns
K48- band 1	1.027	0.967	1.017	significant
K48- band 2	0.993	0.989	1.035	ns
K48- band 3	1.028	0.971	1.011	ns
K48- band 4	1.102	0.889	0.977	significant
K48- band 5	1.012	0.980	1.004	ns
K48- band 6	0.988	1.010	1.008	ns
K48- band 7	1.051	0.952	1.017	ns
K48- band 8	1.018	0.993	0.973	ns

Table 3. *Mean protein levels of free ubiquitin, K48- and K63-linked ubiquitin chains.*

Protein levels of K48 bands 1 (*p=0.040*) and 4 (*p=0.017*) are lower in BD compared to CON. Protein levels are unchanged in SCZ.

Confounders:

- Diagnosis * sex interactions were observed for UBC gene expression, Ub 22kDa, K48 band 1 and K48 band 2.
- UBB gene expression and K63 band 2 correlated with serum CRP levels.
- Psychotropic medications did not significantly impact gene or protein measures.

Conclusion

mRNA expression of UBC, which plays a key role in maintaining cellular ubiquitin levels under stress conditions, is lower in BD and SCZ.

Formation of K-48 ubiquitylated proteins is lower in the prefrontal cortex in BD, potentially suggestive of aberrant protein degradation.

Further investigation of mechanisms underlying dysregulation of the UPS in SCZ and BD may inform future therapeutic interventions in these disorders.

References

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Acknowledgements

Funding for this project was provided by the BC Proteomics Network. CH received a BCMHSUSRI Summer Studentship.

Post-mortem brain tissue samples were donated by the Stanley Medical Institute brain collection.

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