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Blood and brain protein levels of ubiquitin-conjugating enzyme E2K (*UBE2K*) are elevated in individuals with schizophrenia

Hannah Meiklejohn[a,b,1], Md Shaki Mostaid[a,c,l,1], Sandra Luza[a,d], Serafino G. Mancuso[a], Dali Kang[a], Sonny Atherton[a,d], Debora A. Rothmond[e,f], Cynthia Shannon Weickert[a,e,f,m], Carlos M. Opazo[a,d], Christos Pantelis[a,c,d,g,h], Ashley I. Bush[c,d], Ian P. Everall[a,c,d,g,i], Chad A. Bousman[a,c,j,k,l,*]

[a] Melbourne Neuropsychiatry Centre, Department of Psychiatry, The University of Melbourne & Melbourne Health, Parkville, VIC, Australia
[b] Melbourne Medical School, Faculty of Medicine Dentistry and Health Sciences, The University of Melbourne, Parkville, VIC, Australia
[c] The Cooperative Research Centre (CRC) for Mental Health, Victoria, Australia
[d] Melbourne Dementia Research Centre, Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, VIC, Australia
[e] Schizophrenia Research Laboratory, Neuroscience Research Australia, Barker Street, Sydney, Australia
[f] School of Psychiatry, Faculty of Medicine, University of New South Wales, Sydney, Australia
[g] Centre for Neural Engineering, Department of Electrical and Electronic Engineering, The University of Melbourne, Carlton, VIC, Australia
[h] NorthWestern Mental Health, Melbourne, Victoria, Australia
[i] Institute of Psychiatry, Psychology and Neuroscience, King's College London, UK
[j] Hotchkiss Brain Institute, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada
[k] Alberta Children's Hospital Research Institute, Calgary, Alberta, Canada
[l] Departments of Medical Genetics, Psychiatry, and Physiology & Pharmacology, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada
[m] Department of Neuroscience & Physiology, Upstate Medical University, Syracuse, New York 13210, USA

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ABSTRACT

A number of recent studies have suggested the ubiquitin proteasome system (UPS) in schizophrenia is dysfunctional. The purpose of this study was to investigate *UBE2K*, a ubiquitin-conjugating (E2) enzyme within the UPS that has been associated with psychosis symptom severity, in the blood and brain of individuals with schizophrenia. Whole blood and erythrocytes from 128 (71 treatment-resistant schizophrenia, 57 healthy controls) individuals as well as frozen dorsolateral prefrontal cortex (DLPFC) and orbitofrontal cortex (OFC) post-mortem samples from 74 (37 schizophrenia, 37 controls) individuals were obtained. *UBE2K* gene expression was assayed in whole blood and DLPFC samples, whereas protein levels were assayed in erythrocytes and OFC samples. Elevated levels of *UBE2K* mRNA were observed in whole blood of individuals with schizophrenia ($p = 0.03$) but not in the DLPFC, while protein levels were raised in erythrocytes and the OFC ($p < 0.001$ and $p = 0.002$ respectively). Findings were not better explained by age, smoking, clozapine plasma levels or duration of illness. Although blood and brain samples were derived from independent samples, our findings suggest peripheral protein levels of *UBE2K* may serve as a surrogate of brain levels and further supports the notion of UPS dysfunction in schizophrenia. Future studies to determine the pathophysiological effects of elevated UBE2K protein levels in the brain of those with schizophrenia are warranted.

1. Introduction

The ubiquitin proteasome system (UPS) is present in all cells, where it plays a key role in the proper function of several biological processes (e.g. neurotransmitter synthesis and receptor recycling, cytokine production and activation) commonly associated with schizophrenia (Lecker et al., 2006; Lehman, 2009; Petroski, 2008). As such, perturbation of this essential system (e.g., changes in ubiquitination) may have numerous downstream biological consequences that could increase the risk for schizophrenia. Genomic, transcriptomic, and proteomic studies have suggested a dysregulation of the UPS among individuals with schizophrenia in blood and post-mortem human brain

* Corresponding author. 3330 Hospital Drive NW, Department of Medical Genetics, University of Calgary, AB, T2N4N1, Canada.
E-mail address: chad.bousman@ucalgary.ca (C.A. Bousman).
[1] Joint first authors.

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Table 1
Clinical and post-mortem brain cohort characteristics.

Characteristic	Clinical cohort			Post-mortem brain cohort		
	TRS (n = 71)	Control (n = 57)	*p*-value	Schizophrenia (n = 37)	Control (n = 37)	*p*-value
Age, mean (sd) years	40.2 (9.7)	39.5 (10.7)	0.702	51.2 (14.1)	51.1 (14.6)	0.955
Gender, % (n) females	25.4 (18)	22 (38.6)	0.108	35.1 (13)	18.9 (7)	0.116
Ethnicity, % (n) Caucasian	89.9 (62)	50 (87.8)	0.742	97.3 (36)	97.3 (36)	1.0
Current smoker, % (n)	46.5 (33)	21.1 (12)	**0.003****	62.2 (23)	24.3 (9)	**0.014***
RIN, mean (sd)	8.78 (0.34)	8.42 (0.98)	**0.006****	7.27 (0.58)	7.30 (0.57)	0.809
Age of onset, mean (sd) years	22.5 (6.3)	–	–	23.7 (6.1)	–	–
Duration of illness, mean (sd) years	17.1 (8.1)	–	–	27.6 (13.8)	–	–
PANSS scores, mean (sd)	–	–	–	–	–	–
Positive	10.28 (5.87)	–	–	–	–	–
Negative	14.72 (5.48)	–	–	–	–	–
Disorganised	7.54 (2.80)	–	–	–	–	–
Excitement	5.51 (2.20)	–	–	–	–	–
Depression	5.96 (3.01)	–	–	–	–	–
Total	62.45 (14.24)	–	–	–	–	–
Clozapine plasma level, mean (sd) μg/L	431.83 (234.46)	–	–	–	–	–
CPZ equivalents, mean (sd) dose in mg/day	141.90 (287.37)	–	–	691.64 (502.20)	–	–
pH (PFC), mean (sd)	–	–	–	6.6 (0.3)	6.7 (0.3)	0.521
PMI, mean (sd) hours	–	–	–	28 (14)	25 (11)	0.210
Agonal state, % (n) 'excellent'	–	–	–	8.1 (3)	37.8 (14)	**0.006****
Hemisphere, % (n) left	–	–	–	54.1 (20)	37.8 (14)	0.162
Brain volume, mean (sd) grams	–	–	–	1408.05 (165.48)	1438.22 (121.74)	0.375

Abbreviations: TRS = treatment-resistant schizophrenia; RIN = RNA integrity number; PANSS = Positive and Negative Syndrome Scale; CPZ = chlorpromazine; PMI = post-mortem interval; AFS = agonal state. *$p < 0.05$, **$p < 0.01$. Bold signifies that those are statistically significant.

tissue (Altar et al., 2005; Arion et al., 2015; Bousman et al., 2010a, 2010b; Horton et al., 1993; Liu et al., 2017; Middleton et al., 2002; Nishimura et al., 2000; Rubio et al., 2013; Scott et al., 2016; Vawter et al., 2001, 2002). Interestingly, recent findings by our group showed an increase in the levels of ubiquitinated proteins in the blood and brain of individuals with chronic schizophrenia compared to those with recent-onset of the illness and healthy controls (Bousman et al., 2019). However, the mechanism by which this accumulation of ubiquitinated proteins occurs remains to be elucidated.

One potential mechanism for elevated ubiquitinated proteins in schizophrenia is an increase in ubiquitination activity. Ubiquitination of a target protein involves the activation, transfer, and attachment of ubiquitin to target proteins via ubiquitin-activating (E1), -conjugating (E2), and -ligase (E3) enzymes, respectively. Previous post-mortem brain and peripheral blood studies have implicated dysregulation of several of these ubiquitin enzymes in schizophrenia (Middleton et al., 2002; Rubio et al., 2013; Vawter et al., 2002) and bipolar disorder (Ryan et al., 2006). Among these ubiquitination enzymes, the ubiquitin-conjugating enzyme known as *UBE2K* (*HIP2*) is of particular interest. Expression of *UBE2K*'s transcript has been shown to be dysregulated in the brain of individuals with a psychiatric illness (Ryan et al., 2006) and in the blood transcript expression was associated with positive symptom severity in schizophrenia (Bousman et al., 2010b). Furthermore, *UBE2K* is one of the few E2 enzymes capable of conjugating activated ubiquitin at lysine-48 residues, the canonical pathway for proteasomal degradation, and there is evidence that *UBE2K* is able to synthesise polyubiquitin chains in the absence of E3 ubiquitin ligases (Chen and Pickart, 1990; Haldeman et al., 1997; van Nocker and Vierstra, 1993; Wilson et al., 2009; Yao and Cohen, 2000) as well as inhibit proteasome function (Song et al., 2003). Collectively this evidence suggests that an increase in *UBE2K* expression could result in elevated levels of ubiquitinated proteins such as we recently observed among individuals with schizophrenia (Bousman et al., 2019). In fact, *UBE2K's* role in ubiquitinated protein accumulation and aggregation has already been implicated in several other brain disorders including Alzheimer's disease, Parkinson's disease, and Huntington's disease (de Pril et al., 2007; Hegde and Upadhya, 2007; Jia et al., 2012) but whether this is also true in schizophrenia is unknown. Thus, utilizing blood and brain samples from those with and without schizophrenia, we tested the hypothesis that *UBE2K* gene and protein expression would be increased in the blood and brain of those with schizophrenia relative to healthy controls. The rationale for investigating erythrocytes is based on the fact that the UPS is the sole system for protein degradation in erythrocytes due to the loss of all other organelles including autophagosomes during their maturation (Yousefi and Simon, 2009). In addition, we tested a secondary hypothesis that *UBE2K's* peripheral gene and/or protein expression would be positively correlated with symptom severity profiles in individuals with schizophrenia to determine if the correlation we previously observed between *UBE2K* gene expression and positive symptom severity (Bousman et al., 2010b) could be replicated.

2. Materials and methods

2.1. Participants

Seventy-one individuals with schizophrenia were recruited from multiple clinical services and the community in Melbourne, Australia, with an additional 57 unrelated healthy controls matched for age, sex, and socioeconomic status recruited from the general community. All individuals with schizophrenia also met recent criteria for "treatment resistance" (Howes et al., 2016), having exhibited poor functioning and residual symptoms despite adequate trials of at least two antipsychotics before management with clozapine. In addition, frozen post-mortem dorsolateral prefrontal cortex (DLPFC) and orbitofrontal cortex (OFC) tissue from 74 individuals (37 schizophrenia, 37 controls) was obtained from the New South Wales Brain Tissue Resource Centre (Sydney, Australia).

Characteristics of both cohorts are outlined in Table 1. Recruitment and inclusion/exclusion criteria for both cohorts are described briefly in the supplementary material, with further detail regarding ascertainment for the post-mortem cohort published elsewhere (Weickert et al., 2010). All procedures were conducted in accord with principles expressed in the Declaration of Helsinki. The New South Wales Brain Tissue Resource Centre has approval from the ethics committees of Sydney South West Area Health Service (Protocol number: X07-0074) and The University of Sydney (Ref No. 555). The current research was approved and conducted under the guidelines of the Human Research

Ethics Committee at the University of New South Wales (HREC 07261). The treatment-resistant schizophrenia cohort recruitment and procedures were approved by the Melbourne Health Human Research Ethics Committee (MHREC ID 2012.069).

2.2. Clinical measures

All living participants were administered the Mini International Neuropsychiatric Interview (MINI) to confirm the diagnosis of schizophrenia and to rule out current or past psychiatric illness in healthy controls (Sheehan et al., 1998). Clinical symptoms were assessed using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) and scored in accordance with the consensus five-factor (i.e. positive, negative, depressed, excited, disorganised/concrete) PANSS model (Wallwork et al., 2012). Clozapine plasma level was measured in all whole blood samples and current/last chlorpromazine equivalent dosage was calculated for all patients according to existing guidelines (American Psychiatric, 1997; Woods, 2003).

2.3. Tissue collection and processing

Whole blood was collected from living participants after overnight fasting and processed according to a standard blood collection and processing protocol (Mostaid et al., 2017b). Erythrocytes were lysed in NP-40 buffer described below minus 10 mM DTT. The lysates were centrifuged and protein concentrations were determined in the NP-40 soluble fraction by a BCA protein assay kit (ThermoFisher, USA) and then stored at −80 °C until use.

Post-mortem DLPFC and OFC samples were obtained at autopsy and processed as previously described (Sinclair et al., 2012). Samples were homogenized 1/5 (w/v) in 50 mM Tris HCl pH 7.5 containing 1% NP-40 (v/v), 10 mM DTT, NaCl 150 mM, 10 mM N-ethylmaleimide (NEM), protease inhibitors cocktail without EDTA (Roche, USA) and phosphatase inhibitors cocktail (Roche, USA). The homogenized samples were centrifuged at 10,000 g for 10 min at 4 °C. Then supernatants (NP40-soluble fraction) were collected and protein concentrations were determined using the BCA Protein Assay Kit and supernatants were aliquoted and stored at −80 °C until use.

2.4. RNA quantification

UBE2K mRNA levels were quantified in whole blood and post-mortem DLPFC blind to diagnosis. RNA extraction and quantification were performed using PureLink® RNA Mini Kit (ThermoFisher Scientific, Waltham, MA, USA) per standard manufacturer's instructions. Total RNA was reverse-transcribed to cDNA using SuperScript® IV First-Strand Synthesis System (Invitrogen, Foster City, CA, USA) using random hexamers. cDNA (10.25 ng) was used as a template for RT-qPCR using master-mix and gene-specific validated TaqMan® assays (Applied Biosystems, Foster City, CA, USA). Inventoried assays (TaqMan®, Invitrogen, USA) were used for *UBE2K* and four reference genes (β-actin, ACTB; ubiquitin C, UBC; glyceraldehyde-3-phosphate dehydrogenase, GAPDH; and TATA-box binding protein, TBP). See Supplementary Table S1 for a list of probes and primers.

Gene expression levels were determined in duplicate using FAM-MGB TaqMan® gene expression probes (Invitrogen, Foster City, CA, USA) in 192x24 Dynamic Arrays IFC in Fluidigm® BioMark™ HD system (South San Francisco, CA, USA) at the Monash Health Translation Precinct Medical Genomic Facility (Hudson Institute of Medical Research, Clayton, VIC, Australia). In addition, no reverse transcriptase controls and no template controls were included to rule out genomic DNA contamination and reagent contamination, respectively. Normalised relative quantities (NRQ, i.e. $2^{-\Delta Ct}$ where $\Delta C_t = Ct_{\text{(candidate gene)}} - Ct_{\text{(geometric mean of reference genes)}}$) of *UBE2K* were calculated using the geometric mean expression of two reference genes (ACTB and UBC) that did not differ between cases and controls. GAPDH and TBP were not used as reference genes as there was significant difference in their expression by group. This is in keeping with Minimum Information for Publication of RT-qPCR Experiments (MIQE) guidelines (Bustin et al., 2009). Details on methods are described elsewhere (Mostaid et al., 2017a).

2.5. Protein quantification

UBE2K protein levels were quantified in erythrocytes and post-mortem OFC blind to diagnosis. All antibodies used in the current study are listed in Supplementary Table S2. Erythrocyte and post-mortem NP40-soluble fractions (30 µg, for each sample) were submitted to electrophoresis in a 4–12% SDS-polyacrylamide gels for 1 h at 120 V. Then, the proteins were transferred to a PVDF membrane for 1 h at 20 V. The blots were blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween-20 (TBST), and were then incubated over night with the primary antibodies diluted in TBST containing 3% BSA, anti-UBE2K and anti-GAPDH (loading control) at 4 °C. After four washings with TBST, the blots were incubated with conjugated secondary antibodies in TBST and 0.1% SDS for 1 h at 22 °C, protected from light during the incubation. Then, the blots were washed six times with TBST for 5 min each. The blots were finally immersed in PBS and the infrared signal detected in a Li-Cor imager (Li-Cor, Lincoln, USA). Boxes were manually placed around each band of expected molecular weight to obtain integrated intensity values acquired in a Li-Cor imager using the Odyssey 4.0 analytical software. Data expressed as arbitrary unit related to the controls.

2.6. Statistical analyses

Data were analysed using SPSS® Statistics 24 (IBM, USA). The chi-squared test was used to compare categorical variables between groups, while independent samples *t*-tests were used for continuous variables. Adjustments for multiple testing were performed using the Benjamini-Hochberg (B-H) procedure where relevant. Quantile-quantile (Q-Q) plots and the Shapiro-Wilk test were used to assess normality of variable distributions (Supplementary Fig. S1). Grubbs' test was used to identify outliers in each dataset, which were removed before further analyses were performed.

Prior to the main analysis, the following factors were assessed as potential confounders: age, sex, chlorpromazine equivalent dose, clozapine plasma levels, age of illness onset, duration of illness, smoking status, and RNA integrity number (gene expression only). In the post-mortem cohort, tissue pH, post-mortem interval, brain hemisphere, and agonal state were also assessed. Agonal state refers to the specific medical conditions (e.g. coma, hypoxia, seizures) preceding death and duration of the terminal phase (Lipska et al., 2006). A variable was considered a confounder if: 1) it was significantly associated with both the cohort and *UBE2K* mRNA or protein levels (liberal significance value of $p < 0.10$), and 2) adjusting for it in linear regression produced a statistically significant R^2 change (ΔR^2, $p < 0.10$) relative to the model containing just the cohort variable (Vittinghoff, 2012). Amongst cases in the clinical and post-mortem cohorts, a series of regressions were also conducted to determine whether chlorpromazine equivalent dose, clozapine plasma levels, age of illness onset, or illness duration were related to *UBE2K* mRNA or protein expression.

2.7. UBE2K mRNA and protein analysis

In the clinical cohort, generalised linear model was used to test the difference in *UBE2K* mRNA and protein expression between cases and controls adjusting for appropriate covariates (age, gender, RNA integrity number, tobacco smoking, and alcohol use).

In the post-mortem cohort, potential confounders (age, gender, tissue pH, post-mortem interval, smoking and agonal state) were tested for their effect on gene and protein expression. Generalised linear

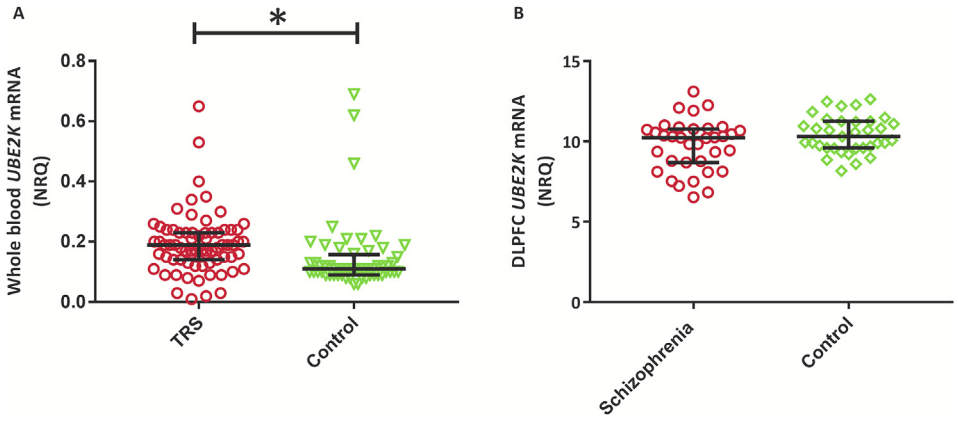

Fig. 1. Normalised relative quantities (NRQ) of *UBE2K* mRNA between schizophrenia and controls in: (A) peripheral blood, from clinical cohort (TRS: 0.19, interquartile range (IQR): 0.14–0.23, controls: 0.11, IQR: 0.09–0.16; Wald chi-square (χ^2) = 5.41, df = 1, p_{adj} = 0.02, $p_{B\text{-}H}$ = 0.03); (B) DLPFC, from post-mortem brain cohort (schizophrenia: 10.23, IQR: 8.77–10.78, controls: 10.31, IQR: 9.60–11.25; Wald chi-square (χ^2) = 4.10, df = 1, p_{adj} = 0.05). Error bars represent median ± interquartile range. TRS = treatment-resistant schizophrenia; DLPFC = dorsolateral prefrontal cortex. *$p < 0.05$.

model was used to assess the difference between groups adjusting for appropriate covariates. All the *p*-values were corrected for multiple comparisons using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995).

For the schizophrenia group within the clinical cohort, Spearman's correlations between mRNA/protein level and symptom severity scores were performed. In addition, mRNA/protein levels between participants in positive symptom remission and those in non-remission were compared using Mann-Whitney *U* test. Positive symptom remission was defined as a score of ≤3 on all four of the PANSS consensus positive symptom items: delusions, hallucinations, grandiosity, and unusual thought content (Wallwork et al., 2012).

3. Results

3.1. UBE2K mRNA expression

UBE2K mRNA levels were found to be significantly elevated in peripheral blood of schizophrenia cases compared with healthy controls (p_{adj} = 0.020; $p_{B\text{-}H}$ = 0.03) (Fig. 1). No significant change in *UBE2K* mRNA levels was observed in the DLPFC in those with schizophrenia, although a trend-level decrease was demonstrated (p_{adj} = 0.05). Importantly, gene expression in schizophrenia cases for both cohorts was not correlated with clozapine plasma levels, chlorpromazine equivalent antipsychotic exposure, age of illness onset, or illness duration. There was also no significant association observed between mRNA levels and age, gender, ethnicity, smoking status, or RIN (Supplementary Tables S3 and S4).

3.2. UBE2K protein expression

Levels of UBE2K protein were shown to be significantly higher in erythrocytes (p_{adj} = 0.00002, $p_{B\text{-}H}$ = 0.00008) and OFC tissue (p_{adj} = 0.001, $p_{B\text{-}H}$ = 0.002) of schizophrenia cases compared with healthy controls (Fig. 2). A significant positive correlation between duration of illness and UBE2K protein expression in the OFC was observed (Spearman's rho = 0.521, $p_{B\text{-}H}$ = 0.028) (Fig. 3A and Supplementary Table S3). There was no association between UBE2K protein levels and chlorpromazine equivalent antipsychotic exposure or age of illness onset for either cohort. However, UBE2K protein levels did differ by smoking status (p = 0.008) and as such is a potential confounder (Supplementary Table S4).

3.3. UBE2K expression and symptomatology

We found a significant negative correlation between erythrocyte UBE2K protein level with positive symptom severity (Spearman's rho = −0.342, p_{raw} = 0.004, $p_{B\text{-}H}$ = 0.048) (Fig. 3B and Supplementary Table S5). Whereas, a significant negative correlation between erythrocyte UBE2K protein levels and disorganised score (r = −0.262, p_{raw} = 0.030, $p_{B\text{-}H}$ = 0.12) and a significant positive correlation between whole blood *UBE2K* mRNA levels and excitement severity score (Spearman's rho = 0.289, p_{raw} = 0.015, $p_{B\text{-}H}$ = 0.090) was also observed (Supplementary Table S5).

Our exploratory analysis of schizophrenia patients in positive symptom remission versus non-remission revealed a statistically significant difference in the levels of erythrocyte UBE2K protein, with greater levels of UBE2K protein in remitters relative to non-remitters (p_{raw} = 0.005, $p_{B\text{-}H}$ = 0.01; Fig. 4). However, no statistically significant difference was observed for *UBE2K* mRNA expression ($p_{B\text{-}H}$ = 0.89).

4. Discussion

We found an elevation of *UBE2K* mRNA levels in whole blood of individuals with schizophrenia but not in the DLPFC. Whereas, UBE2K protein levels were raised in both erythrocytes and the OFC in schizophrenia. Our results, in part, support our primary hypothesis that *UBE2K* mRNA and protein levels would be increased in the blood and brain of those with schizophrenia and suggests that *UBE2K*-mediated ubiquitination may be one of several mechanisms contributing to the accumulation of ubiquitinated proteins, a characteristic we previously observed in the blood and brain of individuals with schizophrenia (Bousman et al., 2019).

Our findings, however, are in contrast with those of the only other independent schizophrenia study of UBE2K protein, which found no change in the level of UBE2K protein in neurons from the superior temporal gyrus (STG) in those with schizophrenia compared with controls (Rubio et al., 2013). There are several possible reasons for these conflicting findings, including the smaller sample size of the STG study (13 schizophrenia, 13 controls), the possibility that UBE2K protein expression in the brain is region- or tissue-specific, or that UBE2K protein expression is age-dependent, a characteristic which was significantly different between the two post-mortem brain cohorts (mean age: current study = 51 years, Rubio et al. = 77 years, $p < 0.0001$). Although age was not associated with UBE2K expression in our study, it is possible that expression of UBE2K decreases in late life. In fact, a decrease in ubiquitin proteasome system functioning is considered one of the hallmarks of aging (Lopez-Otin et al., 2013) and findings presented by Rubio et al. support this notion (Rubio et al., 2013). Thus, our results may not be generalizable to elderly individuals with schizophrenia.

We did not find support for our secondary hypothesis, which was based on our previous findings (Bousman et al., 2010b) that suggested an increase in *UBE2K* gene expression in peripheral mononuclear blood cells (PBMCs) was associated with an increase in positive symptom severity. On the contrary, we found significant negative correlation between UBE2K protein expression and positive symptom severity. Moreover, individuals in positive symptom remission (i.e. lowest

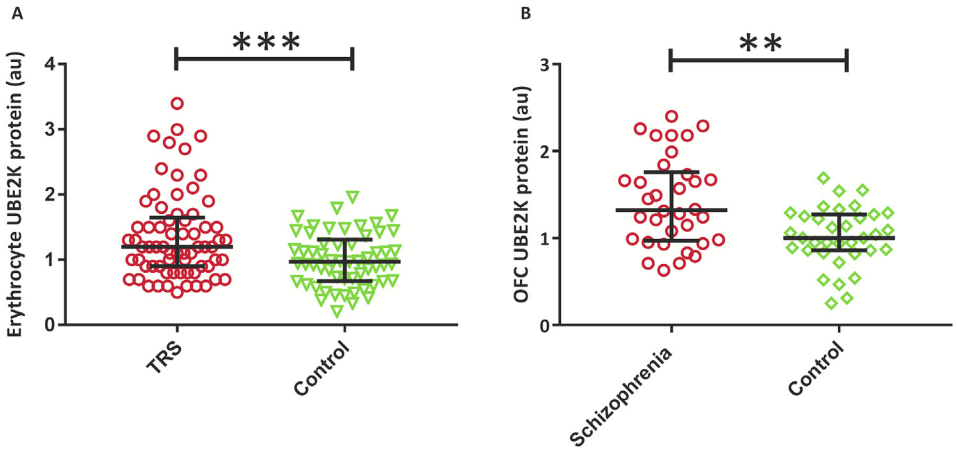

Fig. 2. UBE2K protein expression between schizophrenia and controls in: (A) peripheral erythrocytes, from clinical cohort (TRS: 1.20, IQR: 0.9–1.65, controls: 0.98, IQR: 0.68–1.31, Wald chi-square (χ^2) = 18.08, df = 1, p_{adj} = 0.00002, $p_{B\text{-}H}$ = 0.00008); (B) OFC, from post-mortem brain cohort (schizophrenia: 1.32, IQR: 0.97–1.76, controls: 1.0, IQR: 0.86–1.27, Wald chi-square (χ^2) = 9.33, df = 1, p_{adj} = 0.001, $p_{B\text{-}H}$ = 0.002). Error bars represent median ± interquartile range. To view an example of a Western blot, see Supplementary Fig. S2. OFC = orbitofrontal cortex. $^{**}p < 0.01$, $^{***}p < 0.001$.

symptom severity) had significantly higher protein levels of *UBE2K*, a result opposite to what we had hypothesized. If correct, these results would suggest elevated erythrocyte *UBE2K* protein levels are a potential marker for positive symptom remission in schizophrenia. However, given the discordance between our current and previous results, additional investigation will be required. The discordance between our previous and current results might be a result of differences in the two clinical populations, tissue examined, and/or *UBE2K* measurement. In our previous study (Bousman et al., 2010b), the clinical population included individuals with psychotic symptoms (both schizophrenia and bipolar) and measured *UBE2K* gene expression in PBMCs using microarray technology. Whereas, the current study examined individuals with treatment-resistant schizophrenia and used qPCR and western blots to measure *UBE2K* gene and protein expression in whole blood and erythrocytes, respectively. Thus, our current results do not necessarily invalidate our previous findings but they do suggest the association between *UBE2K* and symptom severity remains tentative and may be highly dependent on the clinical and technical context in which *UBE2K* is examined. Investigations capable of accounting for this variability in clinical populations and measurement are warranted.

Several limitations should be noted. Although this study represents the largest *UBE2K* study in schizophrenia to date, our samples were relatively small and may have limited our power to adequately test our hypotheses. As the study was cross-sectional in design, it was not possible to examine temporal expression patterns and their relationship to clinical course. There was also an absence of reliable clinical data for the post-mortem cohort, which prevented any analysis of *UBE2K* expression and schizophrenia symptomatology for this group. Finally, two different regions of frontal cortex were used to examine *UBE2K* mRNA and protein levels, with mRNA transcripts measured in the DLPFC and protein measured in the OFC. It is therefore difficult to make any broad inferences about the relationship between *UBE2K* gene and protein expression changes in the frontal cortex in schizophrenia.

In summary, this study represents the first investigation of *UBE2K* expression in both peripheral blood and select regions of the frontal cortex in schizophrenia. Our findings add to the general notion that the ubiquitin proteasome system is dysregulated in schizophrenia and more specifically provides evidence to suggest mRNA and protein levels of *UBE2K* are elevated in the blood and brain of individuals with schizophrenia. Importantly, this elevation in *UBE2K* expression provides one potential mechanism by which ubiquitinated proteins accumulate in schizophrenia (Bousman et al., 2019). However, further investigation is required in order to replicate our findings and better characterise the link between *UBE2K* and ubiquitinated protein levels in schizophrenia.

Contributors

Authors CP, IE, AB, and CAB designed the study and wrote protocol. Authors HM and MSM managed the literature searches, conducted the experiments and analyses and wrote the first draft of the manuscript. Authors SL, DK, and SM helped in the experiments and analysis of data. All authors contributed to and have approved the final manuscript.

Conflicts of interest

Dr. Bush is a shareholder in Prana Biotechnology Ltd, Mesoblast Ltd, Grunbiotics Pty/Ltd, Cogstate Ltd, and a payed consultant for and receives profit share remuneration from Collaborative Medicinal Development Pty/Ltd. All other authors reported no competing interests. Dr. Cynthia Shannon Weickert is on an advisory board for Lundbeck Australia Pty Ltd. and in collaboration with Astellas Pharma Inc., Japan.

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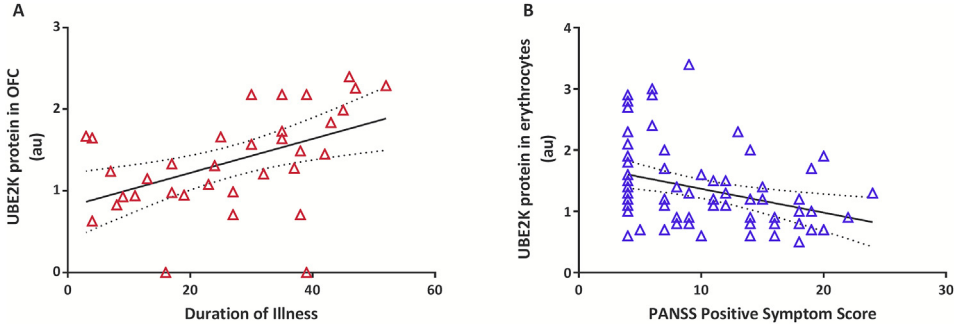

Fig. 3. Correlation between: (A) Duration of illness and OFC UBE2K protein expression from post-mortem brain cohort (Spearman's rho = 0.592, $p_{B\text{-}H}$ = 0.028), (B) Peripheral erythrocytes UBE2K protein level with PANSS positive symptom severity score (Spearman's rho = −0.342, $p_{B\text{-}H}$ = 0.048).

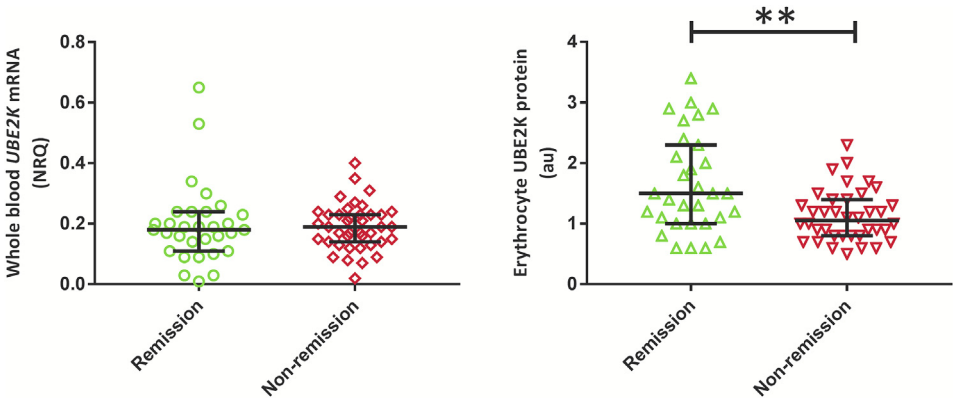

Fig. 4. *UBE2K* expression in peripheral blood, from clinical cohort, by positive symptom remission status: (A) *UBE2K* mRNA expression (remission: 0.18, IQR: 0.11–0.24, non-remission: 0.19, IQR: 0.14–0.23; Wald chi-square (χ^2) = 0.020, df = 1, $p_{B\text{-}H}$ = 0.89 (B) peripheral erythrocytes UBE2K protein expression (remission: 1.50, IQR: 1.0–2.30, non-remission: 1.05, IQR: 0.8–1.40, Wald chi-square (χ^2) = 7.76, df = 1, $p_{B\text{-}H}$ = 0.01). Error bars represent median ± interquartile range. Positive symptom remission was defined as a score of ≤3 on four PANSS items (delusions, hallucinations, grandiosity, and unusual thought content) (Wallwork et al., 2012). **p = 0.01.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2019.03.005.

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