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Cortical copper transporter expression in schizophrenia: interactions of risk gene dysbindin-1

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Abstract

Schizophrenia susceptibility factor dysbindin-1 is associated with cognitive processes. Downregulated dysbindin-1 expression is associated with lower expression of copper transporters ATP7A and CTR1, required for copper transport to the central nervous system. We measured dysbindin-1 isoforms-1A and −1BC, CTR1, and ATP7A via Western blots of the postmortem dorsolateral prefrontal cortex (DLPFC) of schizophrenia subjects (n = 28) and matched controls (n = 14). In addition, we subdivided the schizophrenia group by treatment status and comorbidity of alcohol use disorder (AUD) and assessed the relationships between proteins. Schizophrenia subjects exhibited similar protein levels to that of controls, with no effect of antipsychotic treatment. We observed a shift towards more dysbindin-1A expression in schizophrenia, as revealed by the ratio of dysbindin-1 isoforms. Dysbindin-1A expression was negatively correlated with ATP7A in schizophrenia, with no correlation present in controls. AUD subjects exhibited less dysbindin-1BC and CTR1 than those without AUD. Our results, taken together with previous data, suggest that alterations in dysbindin-1 and copper transporters are brain-region specific. For example, protein levels of ATP7A, dysbindin 1BC, and CTR1 are lower in the substantia nigra in schizophrenia subjects. AUD in the DLPFC was associated with lower protein levels of dysbindin-1 and CTR1. Changes in dysbindin-1 isoform ratio and relationships appear to be prevalent in the disease, potentially impacting symptomology.

Keywords

Postmortem; Cognition; ATP7A; CTR1; Alcohol; Copper

Introduction

Schizophrenia clinically presents in early adulthood with psychotic features as a hallmark symptom. However, cognitive deficits are a core feature of the illness, as they frequently

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Author's contributions Authors Schoonover and Roberts were responsible for the conception, analysis, interpretation, drafting, and revising of the manuscript. Authors Schoonover and Kennedy were responsible for data acquisition. All authors approved the manuscript and are accountable for all aspects of the work discussed herein.

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occur, present before psychosis, and are independent of psychotic symptoms (Keefe and Harvey 2012; Meier et al. 2014). Although not implicated by genome-wide association studies (Ripke et al. 2020; Schizophrenia Working Group of the Psychiatric Genomics 2014), the dystrobrevin binding protein 1 (*DTNBP1*) gene is associated with schizophrenia susceptibility risk (Allen et al. 2008) and the cognitive deficits of the disease (Burdick et al. 2006). Indeed, *DTNBP1* variation is associated with impaired working memory in schizophrenia (Donohoe et al. 2007; Wolf et al. 2011), purportedly due to less prefrontal cortex dysbindin-1 protein and mRNA expression (Tang et al. 2009; Weickert et al. 2004).

Dysbindin-1 has a far-reaching interactome, including somal, cytoskeletal, protein transport, and signal transduction roles (Guo et al. 2009). Of particular interest is that dysbindin-1 modulates copper transporters and thus copper levels via the dysbindin/BLOC-1-copper metabolism interactome (Gokhale et al. 2015; Mullin et al. 2011; Warde-Farley et al. 2010). Knockout of dysbindin-1 in mice results in schizophrenia-like cognitive impairments, such as impaired long term, working, and spatial memory (Cox et al. 2009; Feng et al. 2008; Papaleo et al. 2012; Takao et al. 2008). Dysbindin-1 knockout mice also exhibit 30–50% lower copper transporters *ATP7A* and *SLC31A1*, which produce the proteins ATP7A and CTR1, respectively (Gokhale et al. 2015); together, ATP7A and CTR1 facilitate copper transport into the central nervous system (Eisses and Kaplan 2005; Scheiber et al. 2010; Yamaguchi et al. 1996). Our recent work implicated deficiencies in cellular copper levels and transporter expression in the substantia nigra in schizophrenia (Schoonover et al. 2018), and others have demonstrated lower *DTNBP1* and *SCL31A1* mRNA within both postmortem anterior cingulate cortex and cultured iPS cells of schizophrenia subjects (Roussos et al. 2012; Wen et al. 2014). Reduced copper causes demyelination and schizophrenia-like cognitive deficits, including impaired spatial and working memory (Gokhale et al. 2015; Gregg et al. 2009; Herring and Konradi 2011; Xu et al. 2010; Zhang et al 2008).

Due to the heterogeneity of schizophrenia, division of the cohort by medication history or comorbidities yields more homogeneous subsets of schizophrenia subjects, in turn permitting more specific hypothesis testing. To test for the potential effect of medication treatment, we subdivided the cohort of patients by on- or off-antipsychotic medication (Wessman et al. 2009). Alcohol use disorders (AUDs) are highly comorbid in schizophrenia patients and are associated with greater severity of psychopathology and neurocognitive dysfunction (Yang et al. 2018). Therefore, we subdivided our cohort based on a comorbid diagnosis of AUD or lack thereof. We hypothesized that protein levels of dysbindin-1 isoforms, CTR1, and ATP7A would be lower in the combined schizophrenia group and that the effects would be more significant in patients with this comorbid diagnosis.

Methods

Postmortem brains—We obtained human brains from the Maryland Brain Collection ($n = 41$) and Alabama Brain Collection ($n = 1$) with consent from the next of kin with IRB-approved protocols. Tissue collection was performed by the same group at both locations, limiting any collection site-specific confounding variables. The dorsolateral prefrontal cortex (DLPFC) was dissected from brain slices, frozen, and stored at − 80 °C. Schizophrenia

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cases (n = 28) were compared to matched normal controls (NC, n = 14). Subdivision of the schizophrenia group occurred in two different ways: (1) treatment status, on- (n = 14) or off-medication (n = 14) at time of death and (2) comorbidity of AUD (n = 12) or lack thereof (n = 14). While there was enough clinical information for most cases to determine AUD, we excluded the cases with insufficient information when subdividing the cohort. Placement into the off-medication schizophrenia group required being off any antipsychotic medication (APD) for a minimum of 6 months before death. The era in which these cases were collected was before today's prevalence of polypharmacy. All medicated subjects were on typical APDs.

Cases were selected based on the best match of the demographic factors of age, race, sex, postmortem interval (PMI), pH, and the number of years frozen (Table 1a). Controls and the schizophrenia group as a whole differed in the length of time the samples were frozen (p = 0.013), as did controls and the schizophrenia subgroups divided by comorbidity of AUD (p = 0.02) (Table 1a). However, correlational analyses of the number of years frozen and the measured proteins did not reveal any significant relationships (Table 1b) and do not appear to impact the study results. Exclusionary criteria for schizophrenia subjects and controls were history/evidence of intravenous drug abuse, HIV/AIDS, Hepatitis B, head trauma, comorbid neurological disorders, custodial death, death by fire, unknown next of kin, children, or decomposed subjects. Agonal status has not been shown to affect protein (Stan et al. 2006), and, therefore, is not an issue with the current investigation.

Diagnoses of schizophrenia and AUD were confirmed independently by two psychiatrists based on DSM criteria at the time of diagnosis (DSM-III-R through DSM-IV-TR) using the Structured Clinical Interview for the DSM (SCID). Subject clinical information (such as medication compliance, age of disease onset, AUD, and symptomology) was obtained from autopsy and medical records, in addition to family interviews. Furthermore, we had access to toxicology analysis performed on all subjects obtained from the MBC. All but two subjects had negative drug test results: two schizophrenia patients tested positive for opiates or hallucinogens at the time of death. No subjects used any illicit drugs. Toxicology analysis was not performed on the ABC subject.

Western blotting

Tissue and protein preparation—The dorsolateral prefrontal cortex (DLPFC) was excised from frozen coronal tissue slices, matching sample position between groups. Removal of excess white matter from the blocks provided a pure grey matter sample. Tissue sonication using an ultrasonic probe (Microson Ultrasonic Cell Disrupter XL) in lysis buffer (500ul/0.1 g of human tissue) containing Tris–HCL (pH 8.0), EDTA, sodium chloride, 10% sodium dodecyl sulfate, and a protease inhibitor cocktail (Sigma; P8340) prevents potential nucleic acid interference with protein purification and also releases membrane protein clusters for detergent and enzymatic interaction (Mirza et al. 2007; Speers et al. 2007). Tissue homogenate was centrifuged at 13,500 rpm (46,500 G) for 15 min at 4 °C to obtain total protein extract (Lee et al. 2002). Following collection of the supernatant (total cell lysate), the Lowry method determined protein concentration (Bio-Rad, Hercules, CA, USA; 500-0113, 500-0114).

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Gel electrophoresis and western blotting—Western blots were used to measure ATP7A, CTR1, and dysbindin-1A and −1BC protein levels and performed as previously reported (Schoonover et al. 2018). Samples were heated to 95 °C for 5 min, except for samples intended for ATP7A assay. 4–20% gradient polyacrylamide gels (Lonza, Basel, Switzerland; 58,505) were loaded with 60 μg of protein extract. Proteins were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis at 150 V for 1 h 15 min, and then transferred at 30 V for 21 h onto polyvinylidene fluoride (PVDF) membranes (Bio-Rad, Hercules, CA, USA; 162–0174) at 4°C. Initial analyses determined the optimal antibody concentrations. The following antibodies and concentrations were used: rabbit anti-C-terminal ATP7A, 1:2,000, Aviva Systems Biology (ARP33798_P050); rabbit CTR1, 1:2,000, Novus Biologicals (NB100-402); rabbit anti-Dysbindin-1 (targeting-1A and −1BC), 1:2,000, Abcam (ab133652); and mouse anti-actin, 1:40,000, Millipore (MAB1501). Horse anti-mouse, 1:15,000 (AP-2000–1) and goat anti-rabbit 1:1,000 (AP-1000–1) secondary antibodies conjugated to alkaline phosphatase from Vector Labs were used. Dysbindin-1A and −1C are primarily expressed within the postsynaptic density, whereas dysbindin-1B is localized to synaptic vesicles within the presynaptic axon terminal (Talbot et al. 2011). Each gel contained a mixture of NC and schizophrenia subjects and was performed in duplicate. The membranes were blocked for 1 h in 5% milk in Tris-Buffered Saline with Tween 20 (TBST). Antigen presence was detected by incubating the primary antibody with the PVDF membrane 21 h at 4 °C. The bands were visualized using chemiluminescence (Bio-Rad; 170–5018), exposing Sigma-Aldrich Carestream Kodak BioMax XAR films (166–0760). As described previously (Schoonover et al. 2018), films were scanned at 600 dpi using a flatbed scanner. Optical densities of the bands were measured using Image J-64 freeware (NIH). A step calibration tablet was used to create an optical density standard curve (Stouffer Industries Inc.; Mishawaka, IN, USA; T2120, series #130501) to which each measurement was calibrated. ImageJ was used to perform a background subtraction for each film. All optical density values for each protein were normalized to actin and then to the averaged NC. These values were averaged for duplicate samples. Figure 1a illustrates representative western blots. A blinded experimenter performed all experimental studies and analyses.

Statistical analyses

Data were summarized using means and standard errors (SE). A *t* test or an ANOVA test assessed demographics and tissue quality. A Chi-squared test assessed categorical variables. SPSS detected and removed any potential outliers. SPSS uses a step of 1.5 × IQR (Interquartile range) to identify outliers. Outliers were determined for each protein for each group: (1) NC, (2) SZ, (3) SZ-on APD, (4) SZ-off APD, (5) SZ with AUD, and (6) SZ no AUD. There were three outliers for dysbindin-1BC: two controls and one SZ-On APD. There were no outliers in any of the other proteins that we analyzed. Removing the outliers did not change the significance of the statistical comparisons.

The Shapiro–Wilk and the D'Agostini and Pearson tests assessed normality. If both tests revealed normally distributed data, parametric tests were used (two groups, unpaired *t* test; three groups, one-way ANOVA). If not, non-parametric tests were used (two groups, Mann–Whitney *U* test; three groups, Kruskal–Wallis *H* test). Furthermore, Brown–Forsythe

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and Bartlett's tests detected the presence of significantly different standard deviations, and Welch's tests via Prism 8 were used upon the discovery of different deviations.

Data assessing the effect of a comorbid diagnosis of AUD on protein expression were analyzed using SPSS per availability to RCR. Data were assessed as previously discussed with the following exceptions: the Kolmogorov–Smirnoff test assessed data normality; all data for these two analyses were normally distributed. Following a significant ANOVA test result, Fisher's Least Significant Difference (LSD) test determined specific between-group differences.

Correlational analyses were performed between results and PMI, age, years frozen, and pH to elucidate potential relationships among these variables using Pearson's correlation or Spearman's rho (used only if the data was not normally distributed). Information was not present on enough subjects to test smoking status. Protein levels were correlated with each other to determine if significant relationships existed. A two-correlation coefficient analysis was then performed using VassarStats to determine if protein relationships significantly differed between groups.

Results

Analysis of protein expression

Representative Western blots are shown in Fig. 1a. All proteins were measured at their expected molecular weight. Schizophrenia subjects exhibited similar levels of CTR1, dysbindin-1A, and dysbindin-1BC to that of controls (Fig. 1b).

No effect of treatment status was observed (Fig. 1c). However, when the schizophrenia cohort was divided by AUD, significant between-group differences were observed for levels of dysbindin-1BC ($p = 0.013$) and CTR1 ($p = 0.016$). Schizophrenia subjects with AUD exhibited less dysbindin-1BC ($p = 0.003$) and CTR1 ($p = 0.005$) protein than patients without this comorbidity (Fig. 2).

The ratio of dysbindin-1 isoforms significantly differed between controls and schizophrenia subjects. Control subjects exhibited an approximate 1:1 ratio of the two dysbindin isoforms; in contrast, schizophrenia subjects expressed an elevated dysbindin-1A/1BC ratio ($p = 0.049$) (Fig. 3). The ratio of isoforms was not dependent upon treatment status or AUD (data not shown).

Correlational analyses

The relationship between dysbindin-1A and ATP7A differed significantly between control and schizophrenia subjects. Schizophrenia subjects exhibited a robust negative relationship between dysbindin-1A and ATP7A, whereas controls did not exhibit a statistically significant relationship between the two proteins (Fig. 4). There was no effect of medication status on the dysbindin-1A/ATP7A relationship (data not shown). No other meaningful correlations were observed.

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Discussion

Here, we studied the protein expression of the schizophrenia susceptibility gene dysbindin-1 and copper transporters CTR1 and ATP7A in the postmortem dorsolateral prefrontal cortex of schizophrenia subjects while also investigating the effect of medication status and AUD comorbidity. Surprisingly, the combined schizophrenia cohort exhibited similar protein levels to that of controls, with no impact of antipsychotic treatment. Dysbindin-1A expression was negatively correlated with ATP7A in schizophrenia, while no correlation was present in controls. The schizophrenia cohort exhibited an altered ratio of dysbindin-1A to dysbindin-1BC. Patients with AUD exhibited less dysbindin-1BC and CTR1 than those without AUD.

Dysbindin-1

The current study did not observe lower dysbindin-1 protein in the combined schizophrenia group for either isoform. Notably, we previously found lower dysbindin-1BC in the substantia nigra of schizophrenia subjects (Schoonover et al. 2018). Taken together, these studies indicate regional and isoform specificity of dysbindin-1 abnormalities in schizophrenia. However, the current study used total grey matter homogenate from the dorsolateral prefrontal cortex. Given the complexity of the cortex and the fact that abnormalities in this brain area in schizophrenia are often restrained to specific cortical layers and cell types (Eastwood and Harrison 2005; Garey et al. 1998; Glantz and Lewis 2000; Ohnuma et al. 1998), it is possible that any potential findings were masked by the heterogeneous nature of the composite sample. Indeed, the neocortical areas of schizophrenia subjects exhibit less dysbindin-1 protein in glutamatergic presynaptic terminals, elevated proteolysis of the SNARE protein complex, and hypoactivity in presynaptic glutamatergic terminals [for review, please see (Schoonover et al. 2020)]. Dysbindin-1 is a critical structural component to synaptic vesicles, postsynaptic densities, and microtubules (Talbot et al. 2006, 2004), and an indirect regulator of several homeostatic systems and protein complexes (Guo et al. 2009). It also modulates glutamatergic neurotransmission (Talbot et al. 2004) and interacts with the SNARE protein complex that modulates glutamate release from astrocytes (Numakawa et al. 2004; Zhang et al. 2004). Therefore, future work will seek to investigate the current proteins of interest in laminar- and cell-specific samples.

Some dysbindin-1 alterations in schizophrenia appear to be isoform-specific (Konopaske et al. 2018; Schoonover et al. 2018; Talbot et al. 2011; Tang et al. 2009). However, findings have been variable, even though similar methods were employed and the same brain region was studied. In the present study, we found no change in protein levels of dysbindin-1A in schizophrenia, consistent with other findings in DLPFC (Tang et al. 2009; Konopaske et al. 2018). Tang et al. (2009) found that their schizophrenia cohort had unaltered dysbindin-1B, but had lower levels of dysbindin-1C protein. Konopaske et al. (2018) found elevated dysbindin-1B that was inversely correlated with basilar dendrite length and number of spines per dendrite. In the present study, we measured dysbindin 1BC and found no change. This difference in results could be due to measuring different isoform combinations. The elevated

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levels of dysbindin 1B (Konopaske et al. 2018) and the lower levels of dysbindin-1C (Tang et al. 2009) may mask alterations when measured together as done here.

In addition, we observed a significantly larger ratio of dysbindin-1A to −1BC in the disease, indicating a shift to a higher dysbindin-1A expression that was not calculated in other studies. While the subcellular localization of dysbindin-1 and its isoforms has been well-characterized in normal postmortem hippocampus (Talbot et al. 2006), the only study to assess dysbindin-1 subcellular localization in schizophrenia did not assess isoforms (Talbot et al. 2004). Therefore, while it is tempting to draw conclusions regarding the expression and localization of our altered dysbindin-1 isoform ratio findings, given what we know about normal dysbindin-1 isoform expression within the synapse, further studies are required for a complete interpretation.

Copper transporters

Among its many functions, dysbindin-1 is an upstream modulator of the copper transporters CTR1 and ATP7A (Gokhale et al. 2015), though it is not the only one. Indeed, copper transporter expression is internally controlled in response to systemic and neuronal cellular copper levels (Labbe et al. 1997; Yamaguchi et al. 1993), as well as other trace metals, such as zinc (a transporter of which was recently implicated by schizophrenia genetic studies Perez-Becerril et al. 2014; Scarr et al. 2016). Therefore, although altered dysbindin-1 in schizophrenia and its modulation of copper transporters in mice was the sparking element to study copper transporters in schizophrenia and their potential relationship to dysbindin-1 in the cortex, the reasons for suspected alterations in copper transport in schizophrenia were threefold. First, experimental manipulations that reduce brain copper, such as the copper chelator cuprizone, recapitulate several pathologies and cognitive impairments common in the disease (Acs and Komoly 2012; Gregg et al. 2009; Xu et al. 2010; Yang et al. 2009). Second, schizophrenia subjects exhibit elevated serum copper (Vidović et al. 2013), which we hypothesized may result from faulty copper transport from the blood into the brain. Finally, our previous work indicated a deficiency in cellular copper transport and quantity in the substantia nigra of schizophrenia subjects (Schoonover et al. 2018).

Although not significant, ATP7A expression in schizophrenia subjects was less than that of controls and appeared to have a bimodal distribution; however, neither division by subgroup, nor any demographic variables, explained the distribution (data not shown). Though we did not observe significant differences in protein levels between groups, we observed an altered relationship between dysbindin-1A and ATP7A in schizophrenia subjects compared to controls. Given that loss of dysbindin-1 in knockout mice reduces *ATP7A* mRNA by approximately 30–50%, the isoform-specific inverse relationship in schizophrenia subjects was surprising and perhaps is attributable to the alteration in dysbindin-1 isoform ratio as previously discussed. Specifically, the shift towards a larger dysbindin-1A/1BC ratio in schizophrenia does not equally alter the expression of ATP7A, as it is not the transcription of *DTNBP1* as a whole resulting in a ratio shift but a change in the alternative splicing of the translated protein. Therefore, perhaps, it is some upstream modulator of both *DTNBP1* and *ATP7A* that results in a positive relationship between the two genes, and not the protein translation itself that modulates copper transporter expression.

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Alcohol use disorder comorbidity

Protein levels of dysbindin-1BC and CTR1 were lower in schizophrenia subjects with AUD. Although this suggests that alcohol misuse contributes to impaired copper transport, the mechanism of action has yet to be determined or studied. However, given the prominent role of astrocytes at the blood–brain barrier and the inflammatory effect of alcohol consumption on both neurons and astrocytes (for review, please see (Abrahao et al. 2017), copper transporters may be lower in schizophrenia subjects with AUD as a consequence of alcohol-induced neuroinflammation. Indeed, heavy alcohol exposure results in increased levels of neuroinflammation markers and reduced astrocyte arbor complexity (Gómez et al. 2018), altered transcriptome responses (for review, please see (Abrahao et al. 2017)), and alterations in neuronal firing patterns (Pleil et al. 2015). Therefore, to accurately interpret the results observed here, future study is needed, such as analyses of cohorts with AUD but without schizophrenia.

Limitations

As is typical for postmortem studies, none of our subjects were first episode or antipsychotic-naïve; therefore, our results could be affected by medication history. However, our findings of no effects in our off-drug schizophrenia subjects assuage this potential confound. The medicated subjects in our cohort were all treated with typical antipsychotic drugs of various types. Thus, the potential effect of atypical antipsychotic drugs could not be examined.

We had no information on the duration of AUD in our subjects, so we could not assess the potential impact of AUD duration. Lack of AUD-only subjects is a limitation to this study; unfortunately, AUD is usually comorbid with other disorders, especially mood and psychiatric disorders (Kessler et al. 1996; Regier et al. 1990), and therefore, AUD-only individuals are rare. An in-depth study would include several disorders with and without AUD comorbidity, which is outside of the current manuscript's scope, but is an area of future study.

In addition, we did not assess cellular copper levels, as we did previously (Schoonover et al. 2018), for which the reasons were twofold: considering that our previous findings indicated uniformly lower levels of copper transporters and cellular copper, we did not deem cellular copper assessment necessary following unaltered protein expression assessment in schizophrenia subjects in the current study. Furthermore, we cannot make within-subject comparisons of prefrontal cortex copper transporter expression to substantia nigra expression. The cohort used in the current study differs from that used previously (Schoonover et al. 2018) and these studies were not performed simultaneously. Future work could simultaneously inspect the cellular, laminar, and regional differences of copper transporter expression in schizophrenia in multiple brain regions.

Conclusions

Although we did not observe lower protein expression of dysbindin-1 and copper transporters ATP7A and CTR1 in the entire patient cohort as expected, the current study

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revealed an altered ratio of dysbindin-1 isoforms and relationship of dysbindin-1A to copper transporter ATP7A. There were no differences between patients on- or off-medication and controls. Finally, patients with a comorbid diagnosis of AUD had lower CTR1 and dysbindin-1BC than patients without this comorbidity. As more studies explore dysbindin-1 and copper enzymes, it becomes apparent that abnormalities are brain-region specific in schizophrenia. Together, these findings suggest that although the levels of dysbindin-1 and copper transporter proteins may be unaltered in the prefrontal cortex in schizophrenia, a laminar- and cell-type-specific investigation is needed. Furthermore, the individual expression level needs not to be altered for schizophrenia-specific changes in ratio and relationships, potentially impacting symptomology in schizophrenia.

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Data availability

Research data will be fully available upon request.

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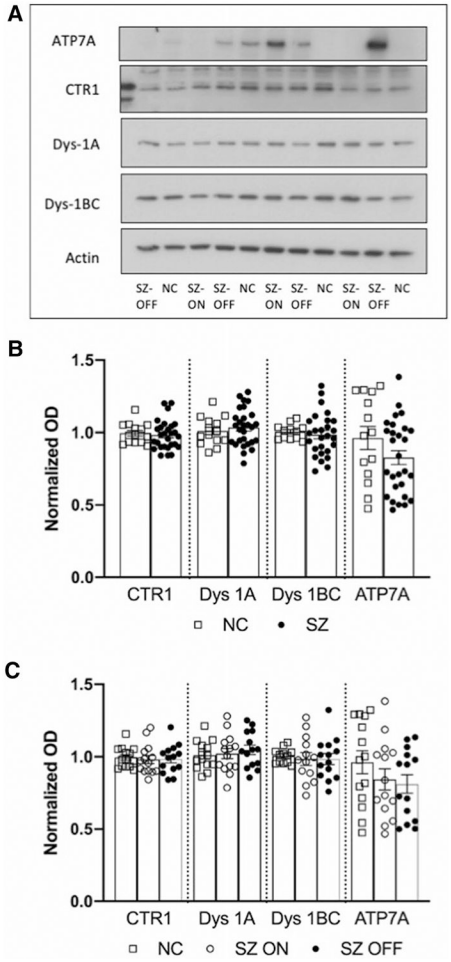

Fig. 1.

a Representative western blots. Actin was the loading control. Analysis of protein expression in controls and subjects with schizophrenia (**b**) and schizophrenia subjects divided by treatment status (**c**)

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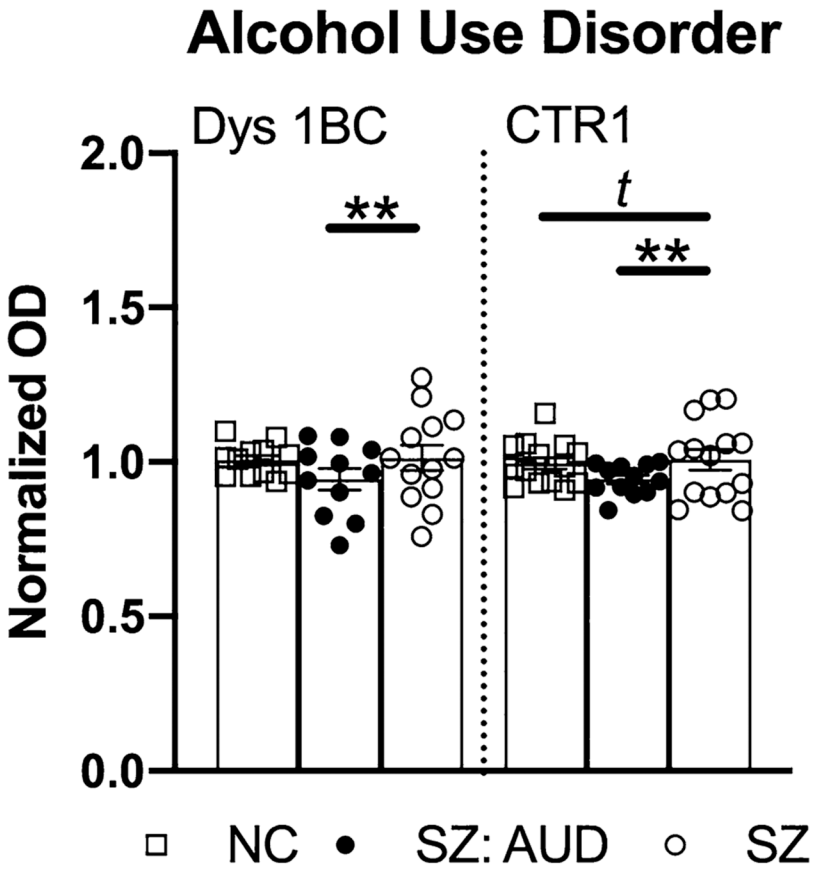

Fig. 2.

ANOVA revealed significant group differences for dysbindin-1BC ($p = 0.013$) and CTR1 ($p = 0.016$). Schizophrenia subjects with AUD exhibited less dysbindin-1BC and CTR1 protein than those schizophrenia subjects without this comorbidity. *$p = 0.05$; **$p = 0.01$

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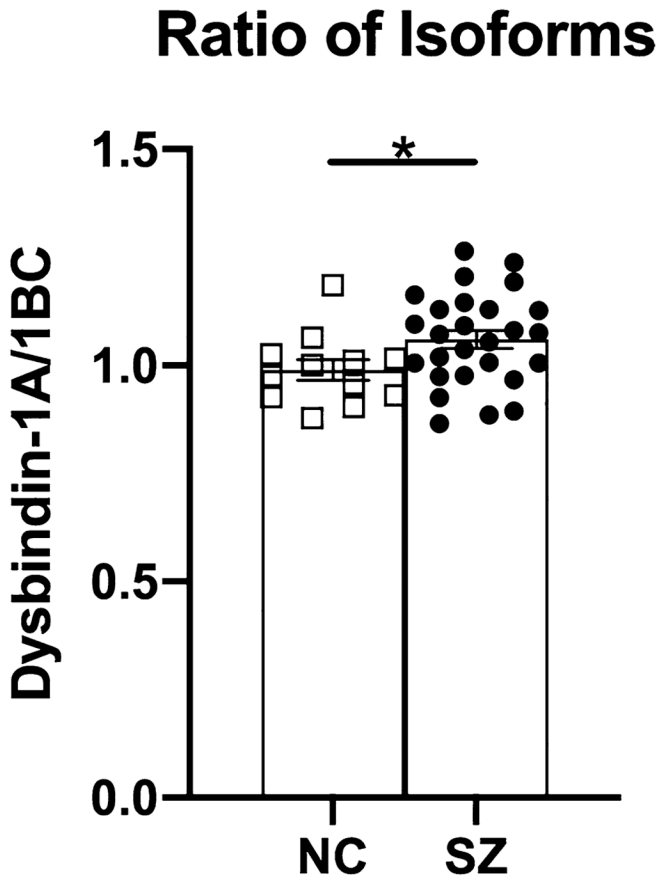

Fig. 3.

Ratio of dysbindin-1 isoforms-1A and −1BC. Dysbindin-1A expression level was divided by dysbindin-1BC expression level within each subject. Schizophrenia subjects exhibited a significantly higher ratio of dysbindin-1 isoforms than controls, indicating a shift towards higher dysbindin-1A expression within the postsynaptic density in schizophrenia. Abbreviations: *NC* normal controls, *SZ* schizophrenia subjects. * indicates a *p* value less than $p = 0.05$

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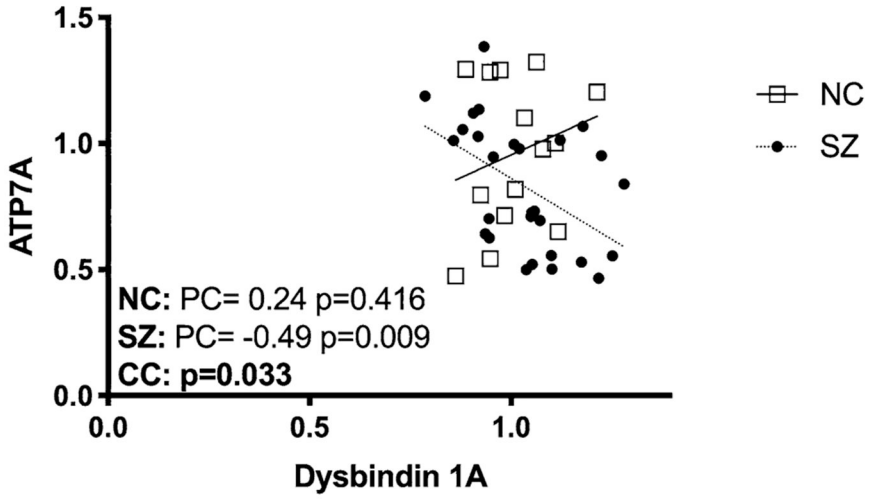

Fig. 4.

Correlational analysis between dysbindin-1A and ATP7A in controls and schizophrenia subjects. A comparison of the two correlation coefficients between schizophrenia subjects and controls was statistically significant ($p = 0.033$)

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Table 1

A) Demographics and markers of tissue quality

(A) Demographics and markers of tissue quality									
Group	**#**	**Age, years**	**PMI, hours**	**pH**	**Years frozen**	**Age of onset, years**	**DUI, years**	**Race**	**Sex**
NC	14	42.3 ± 14.3	13.2 ± 6.3	6.7 ± 0.5	20.9 ± 6.9	N/A	N/A	9C/5AA	10 M/4F
SZ	28	43.3 ± 13.6	15.7 ± 6.7	6.6 ± 0.3	24.9 ± 3.2	25.3 ± 6.4	19.8 ± 12.8	16C/12AA	22 M/6F
Comparison		$p = 0.83$	$p = 0.25$	$p = 0.42$	***p = 0.013***	N/A	N/A	$\chi^2 = 0.66$	$\chi^2 = 0.61$
SZ-On	14	45.0 ± 14.0	16.6 ± 5.9	6.5 ± 0.4	25.7 ± 3.7	24.7 ± 8.1	22.1 ± 11.6	8C/6AA	11 M/3F
SZ-Off	14	41.5 ± 13.4	14.8 ± 7.4	6.6 ± 0.3	25.5 ± 2.7	25.8 ± 3.7	16.2 ± 13.6	8C/6AA	11 M/3F
Comparison		$p = 0.99$	$p = 0.90$	$p = 0.14$	$p = 0.37$	$p = 0.71$	$p = 0.32$	$\chi^2 = 0.91$	$\chi^2 = 0.88$
SZ:AUD	16	44.0 ± 13.3	14.9 ± 6.8	6.5 ± 0.4	25.0 ± 3.7	25.4 ± 3.7	20.9 ± 13.6	9C/7AA	12 M/4F
SZ: no AUD	9	42.6 ± 14.7	18 ± 5.7	6.5 ± 0.3	27.0 ± 1.8	25.0 ± 8.3	17.6 ± 11.9	5C/4AA	9 M/0F
Comparison		$p = 0.91$	$p = 0.23$	$p = 0.37$	***p = 0.02***	$p = 0.90$	$p = 0.59$	$\chi^2 = 0.88$	$\chi^2 = 0.21$

(B) Pearson correlation for years frozen								
Group	**ATP7A**		**CTR1**		**Dysbindin-1A**		**Dysbindin-1BC**	
	PC	***p* value**	**PC**	***p* value**	**PC**	***p* value**	**PC**	***p* value**
NC	− 0.163	0.578	0.255	0.379	− 0.012	0.966	0.061	0.851
SZ	− 0.175	0.374	− 0.008	0.969	0.145	0.460	− 0.075	0.710
SZ-On	− 0.126	0.668	0.038	0.898	0.096	0.744	− 0.117	0.704
SZ-Off	− 0.267	0.356	− 0.075	0.799	0.242	0.405	− 0.012	0.968
SZ: AUD	− 0.106	0.695	− 0.287	0.281	0.109	0.688	− 0.225	0.402
SZ: no AUD	0.097	0.804	0.220	0.571	0.098	0.802	− 0.087	0.825

The number of years a sample was frozen significantly differed between control and subjects with schizophrenia ($p = 0.013$), and among controls and schizophrenia subjects with a comorbid alcohol use disorder ($p = 0.02$). No other significant differences were found. (B) We performed correlations between proteins and the number of years the sample was frozen to determine the impact on protein levels. No significant correlations were observed. All medicated schizophrenia patients were on typical antipsychotic drugs: fluphenazine ($n = 4$), chlorpromazine ($n = 2$), haloperidol ($n = 3$), trifluropromazine ($n = 1$), mesoridazine ($n = 1$), and no information ($n = 3$)

AA African American, *C* Caucasian, *DUI* duration of illness, *F* female, *M* male, *NC* normal controls, *PMI* postmortem interval, *SZ* schizophrenia subjects, *SZ-ON* schizophrenia subjects on medication, *SZ-OFF* schizophrenia subjects off medication, *SZ* AUD schizophrenia subjects with a comorbid alcohol use disorder, *SZ* no AUD schizophrenia subject without an alcohol use disorder. Data are reported as mean and SD. Pearson correlation (PC) and *p* values are shown with two-tailed significance