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# Abnormal Expression of Glutamate Transporter and Transporter Interacting Molecules in Prefrontal Cortex in Elderly Patients with Schizophrenia

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## Abstract

Glutamate cycling is critically important for neurotransmission, and may be altered in schizophrenia. The excitatory amino acid transporters (EAATs) facilitate the reuptake of glutamate from the synaptic cleft and have a key role in glutamate cycling. We hypothesized that expression of the EAATs and the EAAT regulating proteins ARHGEF11, JWA, G protein suppressor pathway 1 (GPS1), and KIAA0302 are altered in the brain in schizophrenia. To test this, we measured expression of EAAT1, EAAT2, EAAT3, and EAAT interacting proteins in postmortem tissue from the dorsolateral prefrontal and anterior cingulate cortex of patients with schizophrenia and a comparison group using in situ hybridization and Western blot analysis. We found increased EAAT1 transcripts and decreased protein expression, increased EAAT3 transcripts and protein, and elevated protein expression of both GPS1 and KIAA0302 protein. We did not find any changes in expression of EAAT2. These data indicate that proteins involved in glutamate reuptake and cycling are altered in the cortex in schizophrenia, and may provide potential targets for future treatment strategies.

### Keywords

GPS1; anterior cingulate cortex; dorsolateral prefrontal cortex; postmortem; Western blot; in situ hybridization

## Introduction

Glutamate is rapidly removed from the synapse by plasma membrane excitatory amino acid transporters (EAATs), primarily localized to postsynaptic neurons and astrocytes (Masson et al., 1999). EAATs are critical for glutamate transmission, because it is reuptake and not

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enzymatic breakdown that is responsible for clearance of glutamate from the synapse (Danbolt, 2001). Of these transporters, EAAT1-3 are expressed abundantly throughout the CNS, while EAAT4 is expressed primarily in the cerebellum, and EAAT5 is found in the retina (Yamada et al., 1996; Arriza et al., 1997). EAAT1 and EAAT2 are generally localized to the plasma membranes of glial cells, and are responsible for the majority of glutamate reuptake in the forebrain (Lehre et al., 1995; Rothstein et al., 1996; Tanaka et al., 1997; Williams et al., 2005). EAAT3 is localized to pre- and postsynaptic neurons in the plasma membrane and cytoplasm, and has been implicated in the regulation of synaptic plasticity (Crino et al., 2002; Levenson et al., 2002).

The EAATs are regulated by a variety of mechanisms, including phosphorylation, glycosylation, enzymatic degradation, and protein-protein interactions. A number of EAAT interacting proteins have been identified, including G-protein suppressor pathway 1 (GPS1), JWA, ARHGEF11, and KIAA0302 (also called beta III spectrin). GPS1 interacts with GLT-1 (the rodent isoform of EAAT2), EAAC1 (EAAT3), and rodent EAAT4. GPS1 decreases GLT-1 mediated glutamate reuptake through a direct protein-protein interaction (Watanabe et al., 2003). JWA is the human homolog of GTRAP3-18, which interacts with and downregulates EAAC1 mediated glutamate reuptake (Lin et al., 2001). GTRAP41 (the rodent isoform of ARHGEF11) and GTRAP48 (the rodent isoform of KIAA0302) interact with rodent EAAT4, increasing glutamate transport (Jackson et al., 2001).

Disruptions in cortical functioning and glutamate transmission have both been implicated in schizophrenia (Itil et al., 1967; Perry, 1982; Bjerkenstedt et al., 1985; Gattaz et al., 1985; Aanonsen and Wilcox, 1986; Korpi et al., 1987; Alfredsson and Wiesel, 1989; Deutsch et al., 1989; Macciardi et al., 1990; Javitt and Zukin, 1991; Tamminga et al., 1992; Saransaari et al., 1993; Choe et al., 1994; Krystal et al., 1994; Lahti et al., 1995; Lehre et al., 1995; Coyle, 1996; Utsunomiya-Tate et al., 1996; Deicken et al., 1997; Goff and Wine, 1997; Omori et al., 1997; Bertolino et al., 1998; Kegeles et al., 1998; Ohnuma et al., 1998; Cecil et al., 1999; Tamminga, 1999; Callicott et al., 2000; Dracheva et al., 2001; Meador-Woodruff et al., 2001; Smith et al., 2001a; Steel et al., 2001; Varoqui et al., 2002; Burbaeva et al., 2003; Hisano, 2003; Manoach, 2003; Ghose et al., 2004; Quintana et al., 2004; Dracheva et al., 2005; Liu et al., 2006). A genetic variant of EAAT1 has been associated with schizophrenia, and expression of a high-risk for schizophrenia allele of the GRM3 metabotropic glutamate receptor is associated with decreased EAAT2 mRNA expression in human prefrontal cortex (Egan et al., 2004; Walsh et al., 2008). Because the glutamate transporters maintain extracellular glutamate, we hypothesized that EAAT1–3 expression is altered in the dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) in schizophrenia. We also predicted that expression of molecules regulating EAAT function, including ARHGEF11, JWA, GPS1, and KIAA0302, are altered in schizophrenia. To test these hypotheses, we investigated both the transcript and protein expression of these EAATs and EAAT interacting proteins in schizophrenia using in situ hybridization and Western blot analysis.

## Materials and Methods

### Subjects

Two groups of subjects provided separately from the Mount Sinai Medical Center Brain Bank were studied (Table 1 and Table 2) for mRNA and protein expression, respectively. 11 subjects (3 control subjects and 8 subjects with schizophrenia) were common to both groups. Neuropathological examination revealed no neurodegenerative disorders including Alzheimer's Disease in any subject. Brain samples were prepared as previously described (Oni-Orisan et al., 2007). ACC was dissected at the level of the genu of the corpus callosum. Tissue blocks were dissected from the dorsal surface of the corpus callosum extending 12–15

mm dorsally and extending 12–15 mm laterally from the midline. DLPFC was dissected as described by Rajkowska and Goldman-Rakic (Rajkowska and Goldman-Rakic, 1995).

### In Situ Hybridization

mRNA expression was measured using in situ hybridization for the following subclones: GPS-1 (Genebank accession no. **BC064503**; nucleotide coding region 1396-1602), ARHGEF11 (**NM_014784**; 1935-2309), JWA (**NM_006407**; 181-520), KIAA0302 (**AB008567**; 5958-6345), EAAT1 (**U03504**; 5260825), EAAT2 (**NM004171**; 601-1026), and EAAT3 (NM004170; 156-979), GLAST (rat EAAT1, **X63744 S49018**; 569-1001) as previously described (McCullumsmith and Meador-Woodruff, 2002; Huerta et al., 2006). We have previously shown specificity of the probes for EAAT1, EAAT2, and EAAT3 using sense and antisense probes (McCullumsmith and Meador-Woodruff, 2002). Specificity of the probes for GPS1 and KIAA0302 is shown in Figure 3.

### Western Blot Analysis

Western Blot analysis was performed as described previously (Oni-Orisan et al., 2007). For each assay, protein was transferred to membranes from multiple gels together in the same apparatus, and subsequent treatments were performed in parallel under identical conditions to minimize interblot variability. Membranes were incubated in blocking solution (5% milk powder in Tris buffered saline with 0.1% Tween (milk/TBST) for GPS1 and KIAA0302, 5% milk powder in phosphate buffered saline with 0.1% Tween (milk/PBST) for EAAT1 and EAAT2, and 3% milk/TBST for EAAT3) for 1 hour at room temperature. Membranes were incubated in primary antibody diluted 1:1000 for GPS1 (Abcam, ab4535), EAAT1 (Santa Cruz, sc-7758), and EAAT2 (Chemicon, AB1783), 1:200 for KIAA0302 (Santa Cruz, sc-28273) 1:250 for EAAT3 (Alpha Diagnostics, EAAC11-A), and 1:10,000 for beta-tubulin (Upstate, 05-661) in blocking solution overnight at 4°C. The GLT-1 antibody was raised to a peptide sharing 100% sequence homology with human EAAT2, and cross-reacts with human EAAT2 (Lauderback et al., 2001). The EAAC1 and spectrin β III antibodies were raised to peptides sharing 100% sequence homology with human EAAT3 and KIAA0302. Membranes were washed in TBST or PBST, then incubated for 1 hour with horseradish peroxidase (HRP) coupled secondary antibody diluted 1:5000 in blocking solution for EAAT1, GPS1, KIAA0302, and beta-tubulin, 1:4000 for EAAT2, and 1:400 for EAAT3. Membranes were washed in TBST or PBST followed by high purity water. Prior to examining protein expression, we tested our EAAT1, EAAT2, EAAT3, GPS1, KIAA0302, and beta-tubulin Western blot assays using varying concentrations of total protein of human cortical tissue homogenate. These control studies demonstrated that our assay was linear for the protein concentrations used in our studies.

### Statistical Analysis

Values from two sections (for in situ hybridizations) or bands (for Western blots) per subject for each region were averaged and converted to optical density. For in situ hybridizations, background from white matter (EAAT1, EAAT2, EAAT3, ARHGEF11, KIAA0302, and JWA) or slide background (GPS1) was subtracted from cortical expression grayscale values before conversion to optical density. For Western blots, optical density was divided by optical density of beta-tubulin from the same lane as a loading control and analyzed as a ratio. Outliers, defined as values more than 2 standard deviations from the mean, were excluded. Because tissue pH is a predictor of RNA integrity, correlation analysis was performed to investigate possible associations between transcript expression and tissue pH (Stan et al., 2006). Protein integrity is generally not affected by pH (Stan et al., 2006). Diagnostic groups were matched for age, pH, and PMI, and t-tests revealed no statistically significant differences between groups for any of these variables. When significant associations between transcript expression and pH

were found, analysis of covariance (ANCOVA) was utilized; otherwise analysis of variance (ANOVA) was utilized with diagnosis as the independent variable and optical density or optical density ratio as the dependent variable. For all tests $\alpha = 0.05$.

## Results

### Transcript Studies

Using in situ hybridization, transcript expression of EAAT1-3, ARHGEF11, GPS1, JWA, and KIAA0302 were measured. There were no associations between EAAT1, EAAT2, ARHGEF11, or KIAA0302 transcript expression and pH in either the ACC or the DLPFC. Correlation analysis revealed an association between EAAT3 transcript expression and pH in ACC ($R= 0.49$, $p < 0.05$) but not DLPFC. Correlation analysis revealed an association between JWA transcript expression and pH in ACC ($R = 0.45$, $p < 0.05$) but not DLPFC.

EAAT1 transcript expression was increased in schizophrenia in ACC ($F(1,21) = 4.54$, $p < 0.05$), but not DLPFC (Figure 1). EAAT3 transcript expression was increased in schizophrenia in ACC ($F(1, 19) = 18.07$, $p < 0.05$), but not DLPFC (Figure 1). JWA transcript expression was increased in schizophrenia in ACC ($F(1,24) = 5.73$, $p < 0.05$). KIAA0302 transcript expression was increased in schizophrenia in ACC ($F(1,24) = 8.59$, $p< 0.05$), but not DLPFC. There were no significant differences between groups for EAAT2, ARHGEF11, or GPS1 (Figure 1 and Figure 2). Sense controls confirmed the specificity of our antisense probes for KIAA0302 and GPS1 (Figure 3). Emulsion dipping in the vicinity of layer V of the ACC confirmed the specificity of EAAT2 expression to small cells, consistent with astrocytic expression (Figure 4).

### Protein Studies

Using Western blot analysis, protein expression of EAAT1-3, GPS1, and KIAA0302 were measured. For EAAT1–3, protein bands were present for both monomeric and multimeric forms (Figure 5). Each of these bands was analyzed separately and in sum. Decreased total EAAT1 expression was found in the DLPFC ($F(1,14) = 6.25$, $p < 0.05$), associated with decreased expression of the monomer ($F(1, 15) = 7.31$, $p < 0.05$), but not the multimer (Figure 6). There were no changes in EAAT1 protein expression in the ACC. There were no changes in EAAT2 protein expression in the DLPFC or ACC (Figure 6). Increased expression of the EAAT3 multimer was found in the ACC ($F(1, 16) = 4.92$, $p < 0.05$) but not the DLPFC in schizophrenia (Figure 6). There were no changes in expression of the monomer or the total amount of EAAT3 protein.

GPS1 protein expression was increased in the ACC in schizophrenia ($F(1, 17) = 6.41$, $p < 0.05$) (Figure 3), but unaltered in the DLPFC. KIAA0302 protein expression was increased in the DLPFC ($F(1, 16) = 10.56$; $p < 0.05$), but not the ACC, in schizophrenia (Figure 6).

### Sex Effects

There was a sex imbalance between diagnostic groups, so the dependent variables that differed by diagnosis were tested for sex effects. There were generally no sex effects for these variables with the exception of EAAT3 transcripts. EAAT3 transcript expression was increased in male subjects compared to female subjects in the ACC ($F(1,20) = 11.07$, $p < 0.05$). When split by sex, EAAT3 transcript expression was increased in males ($F(1,8) = 15.04$, $p < 0.05$) and females ($F(1,10) = 5.04$, $p < 0.05$) with schizophrenia compared to same sex controls.

### Antipsychotic Effects

A number of patients with schizophrenia were medication-free for at least 6 weeks prior to death. No differences in transcript expression were detected between medicated and

unmedicated patients for most of the transcripts found altered with the exception of JWA. An increase in JWA expression in the ACC was found in medicated versus unmedicated patients ($F(1,4) = 13.13$, $p < 0.05$). No changes in transcript expression detected between control subjects and unmedicated patients with schizophrenia for any of the transcripts found altered in schizophrenia. No differences in protein expression were detected between medicated and unmedicated patients with schizophrenia for any of the proteins found altered in schizophrenia. No changes in protein expression were detected between control subjects and unmedicated patients with schizophrenia for EAAT1, GPS1, or KIAA0302. EAAT3 protein expression was increased in the ACC in unmedicated patients with schizophrenia compared to controls 10 ($F(1, 9) = 7.07$, $p < 0.05$).

## Discussion

We found alterations in expression of molecules involved in glutamate transport in schizophrenia, suggesting abnormal glutamate reuptake is involved in the pathophysiology of this illness. Of particular interest are the changes in protein expression of the glial transporter EAAT1, the neuronal transporter EAAT3, and the negative modulator of EAAT2 mediated glutamate reuptake, GPS1. These results suggest that neuronal glutamate reuptake may be increased in schizophrenia, while glial glutamate reuptake may be diminished.

We detected a decrease in total EAAT1 protein expression in the DLPFC, most likely attributable to decreased expression of the monomer. While the EAATs function natively as homomultimers (Haugeto et al., 1996), this result suggests that there may be a decrease in monomeric pools of EAAT1. Decreased EAAT1 protein expression may reflect decreased glutamate reuptake into glial cells or a decrease in the total number of EAAT1 expressing glia. In addition to decreased protein expression in the DLPFC, we detected increased EAAT1 transcript, but not protein, expression in the ACC. Unchanged protein expression with increased mRNA expression may be due to a number of factors including abnormal translation and folding, abnormal post-translational modifications, or increased protein turnover.

We did not detect changes in transcript or protein expression of EAAT2, the transporter responsible for approximately 90% of glutamate reuptake in the forebrain (Robinson, 1998). Previous studies of EAAT2 expression have yielded discrepant results. Two studies found increased EAAT2 transcript expression in the PFC, while another found no changes (Ohnuma et al., 1998; Matute et al., 2005; Lauriat et al., 2006). While we did not find changes in EAAT2, it is possible that EAAT2 mediated glutamate reuptake is regulated by processes other than simply increasing or decreasing gene expression. Phosphorylation of specific serine residues and degradation pathways are known to influence EAAT2 function (Kalandadze et al., 2002; Boston-Howes et al., 2006).

EAAT2 mediated glutamate reuptake may also be regulated by GPS1 which interacts with the C-terminus of GLT-1 (the rat homolog of EAAT2), regulating surface trafficking through a leucine zipper-like motif (Watanabe et al., 2003). Coexpression of GPS-1 with GLT-1 downregulates glutamate reuptake in HEK cells (Watanabe et al., 2004). We found increased GPS1 protein, but not mRNA, in the ACC, suggesting decreased EAAT2 mediated glutamate reuptake. GPS1 protein levels have not previously been examined in schizophrenia, but in a different study (Huerta et al., 2006), we found no changes in GPS1 mRNA expression in the thalamus, suggesting that changes in GPS1 may be region specific.

GPS1 may have other functions besides regulating GLT-1/EAAT2. GPS1 also associated with EAAC1 and rodent EAAT4 in an in vitro binding assay, and it might regulate these transporters in a manner similar to GLT-1 (Watanabe et al., 2003). It has also been suggested that GPS1 functions in humans as a suppressor of G-protein pathway signaling (Spain et al., 1996). Thus,

it is possible that GPS1 could also have effects on G-protein linked receptors, including the mGluRs. mGluRs play a role in glutamate cycling because activation of group I mGluRs or group II-III mGluRs enhances and inhibits glutamate release, respectively (Cartmell and Schoepp, 2000).

While it appears that EAAT2 is not regulated simply by changes in gene expression, EAAT3 expression was increased at both transcript and protein levels. Animal and cell culture experiments have shown that the EAATs exist natively as homomultimers (Haugeto et al., 1996). EAAT trimers are formed immediately after biosynthesis, and are the only functioning forms of EAAT3 in native systems (Gendreau et al., 2004). It is unclear whether the multimer bands we measured are dimers or trimers, given that undissociated proteins do not migrate with a uniform surface area to charge ratio (van Holde, 1985). Our data suggest increased EAAT3 mediated glutamate reuptake in the ACC in schizophrenia, since we found an increase in multimer protein expression.

We propose that the changes in EAAT3 expression are secondary to changes in EAAT1 and GPS1 expression. Decreased EAAT1 and increased GPS1 expression may cause decreased glutamate reuptake in glial cells, leading to increased basal synaptic glutamate levels and synaptic spillover. EAAT3 is largely localized in perisynaptic regions and contributes to clearance of glutamate spillover (Diamond, 2001; Huang and Bergles, 2004). Thus, EAAT3 expression may be increased in neurons to compensate for these changes. Consistent with this notion, EAAT3 protein is localized to regulatable cytosolic pools that may be rapidly mobilized to the plasma membrane, suggesting a biological process that is highly responsive to changes in glutamate levels (Davis et al., 1998). It is also possible that increased EAAT3 expression is an effect of direct interaction with GPS1, or another EAAT regulating molecule. However, it is not known what effect, if any, GPS1 has on EAAT3 mediated glutamate reuptake.

In addition to the changes in GPS1, EAAT1, and EAAT3, we detected increased KIAA0302 protein expression in the DLPFC. This increase is difficult to interpret as it relates to glutamate transport because KIAA0302 is reported to interact only with EAAT4 (Jackson et al., 2001). Although both transcript and protein levels of KIAA0302 are highly expressed in the cortex, EAAT4 is expressed at very low levels, and is predominantly expressed in the cerebellum (Nagao et al., 1997; Lin et al., 1998). This anatomical mismatch suggests that KIAA0302 has other functions. KIAA0302 is a structural molecule involved in Golgi and vesicular membrane skeletons, binds to dynein and dynactin, and it has been implicated in vesicular trafficking along microtubules (Stankewich et al., 1998; Holleran et al., 2001; Jackson et al., 2001). Given the scarcity of EAAT4, it is likely that KIAA0302 serves structural, trafficking, or other roles in the prefrontal cortex.

We did not detect changes in transcript expression for any of the EAAT interacting proteins that we studied. This suggests that these molecules are not altered in schizophrenia at the mRNA level. We were unable to measure protein expression of JWA or ARHGEF11 with commercially available antibodies. It is therefore possible that, similar to GPS1 and KIAA0302, they are altered in schizophrenia at the level of protein, but not mRNA, expression.

Another point of interest from these data is that all significant changes were detected in the anterior cingulate but not the dorsolateral prefrontal cortex. Although the DLPFC has been a major focus of studies involving altered gene expression in schizophrenia, many other regions have also been implicated in this disease, and may have more robust changes. In a microarray study of multiple brain regions in schizophrenia, the anterior cingulate cortex had ~20 fold more altered genes than the dorsolateral prefrontal cortex. (Katsel et al., 2005) Thus, our findings are consistent with the hypothesis that the anterior cingulate cortex may be a particularly vulnerable site in the pathophysiology of schizophrenia.

There are several potential limitations of this study. One concern is the advanced age of the subjects. Disorder-specific alterations in gene expression could be masked by age effects on those genes. However, we have previously detected robust changes in glutamate receptor transcripts, proteins, and binding sites in a similar sample from the same brain bank, demonstrating significant changes in gene expression in older subjects (Smith et al., 2001a; Smith et al., 2001b; Clinton et al., 2003; Bruneau et al., 2005; Huerta et al., 2006). Sex effects may also be a confounding factor. In our entire sample, we found an increase in EAAT3 transcript expression in males compared to females in the ACC. However, EAAT3 expression was increased in schizophrenia compared to controls in both males and females when analyzed separately, suggesting that our diagnosis-related results are likely not attributable to sex differences.

Another limitation of this study is the potential effects of treatment with antipsychotic medications (De Souza et al., 1999; Melone et al., 2001). We generally did not detect any differences in expression between medicated and unmedicated patients for any transcripts or proteins we found increased in schizophrenia with one exception. We detected increased JWA transcript expression in the ACC in medicated versus unmedicated subjects. These analyses, however, are underpowered. The increased JWA transcript expression in schizophrenia may therefore be due to either disease specific or medication specific effects. However, we did find increased EAAT3 protein expression in the ACC in unmedicated patients with schizophrenia, compared to control subjects, suggesting that our EAAT3 findings are not due to a medication effect.

Our results shed light on several aspects of glutamate cycling in schizophrenia. Increased GPS1 protein and decreased EAAT1 protein suggest decreased glutamate reuptake capacity in glia, leading to increased synaptic glutamate levels and synaptic spillover. The increase in EAAT3 expression might be compensatory to decreased glutamate reuptake by glia. This scenario suggests a state of increased basal synaptic glutamate levels in the prefrontal cortex in schizophrenia. It remains unclear how KIAA0302 fits into this model, as the transporter subtype it regulates is expressed at very low levels in the cortex. These findings have important implications for neuronal plasticity in schizophrenia, and provide new targets for the development of novel treatments for this illness.

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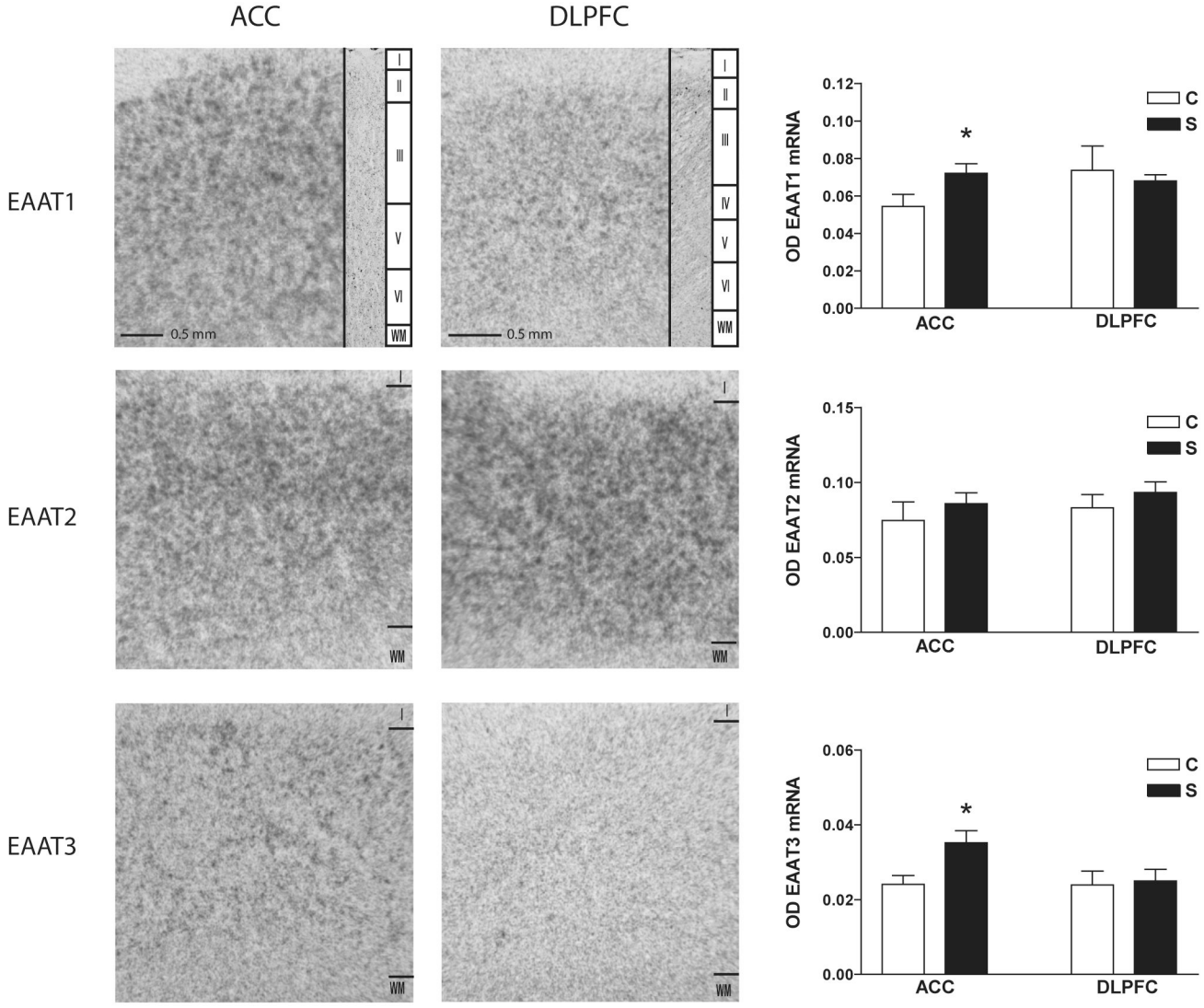


**Figure 1. EAAT transcript expression**

In situ hybridization analysis of EAAT1-3 mRNA expression in the anterior cingulate cortex and dorsolateral prefrontal cortex from control and schizophrenia subjects. Nissl stained sections are shown for comparison. Roman numerals indicate cortical layers. Data expressed as means +/− standard error of the mean. Asterisks indicate a significant difference between control and schizophrenia ($p < 0.05$). Abbreviations: Optical density (OD), control (C), schizophrenia (S), anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DLPFC), excitatory amino acid transporter (EAAT), layer I (I), white matter (WM).

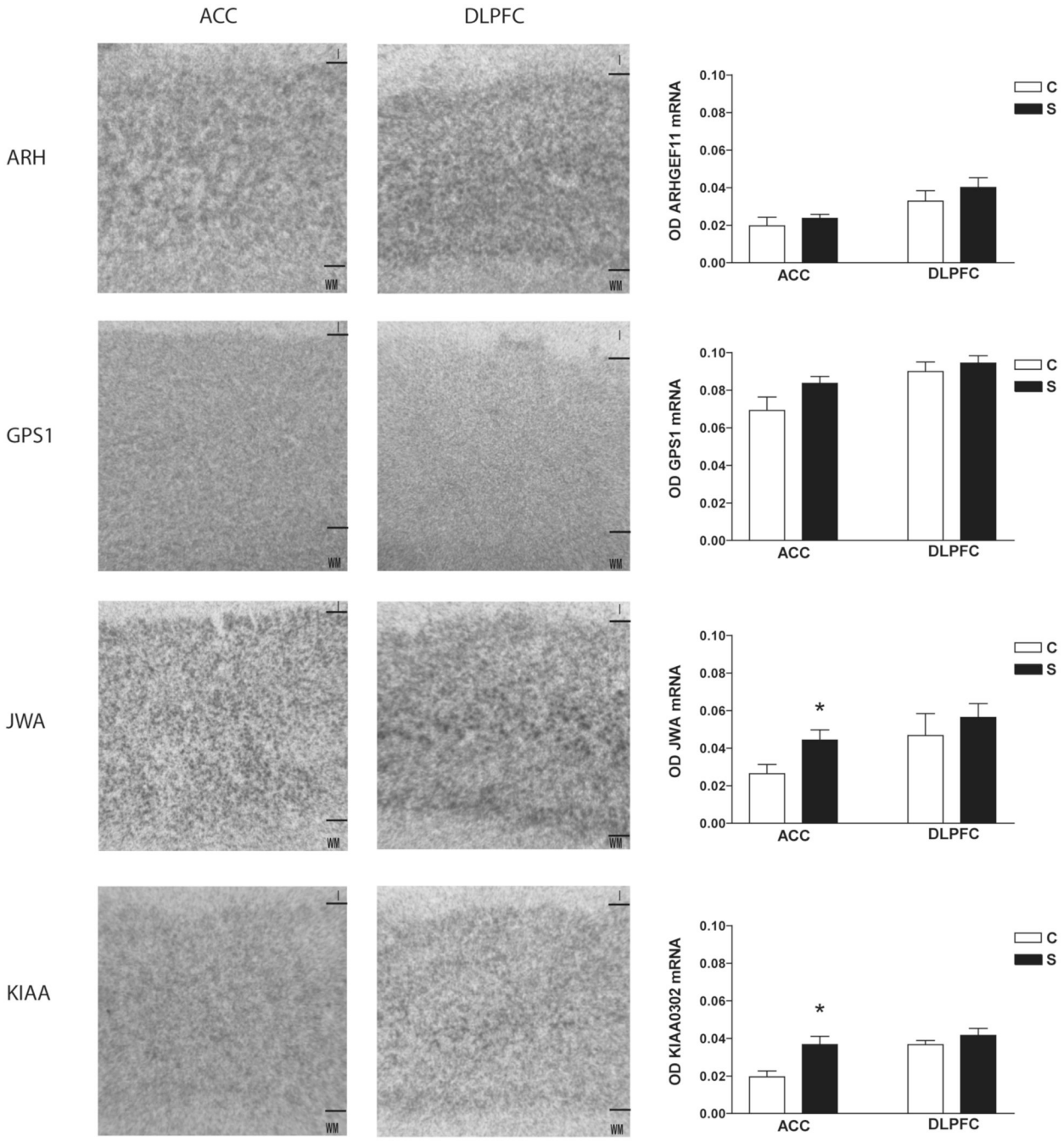


**Figure 2. EAAT interacting partner transcript expression**

In situ hybridization analysis of ARHGEF11, GPS1, JWA, and KIAA0302 mRNA expression in the anterior cingulate cortex and dorsolateral prefrontal cortex from control and schizophrenia subjects. Data expressed as means +/− standard error of the mean. Abbreviations: Optical density (OD), control (C), schizophrenia (S), anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DLPFC), ARHGEF11 (ARH), G-protein suppressor pathway 1 (GPS1), KIAA0302 (KIAA), layer I (I), white matter (WM).

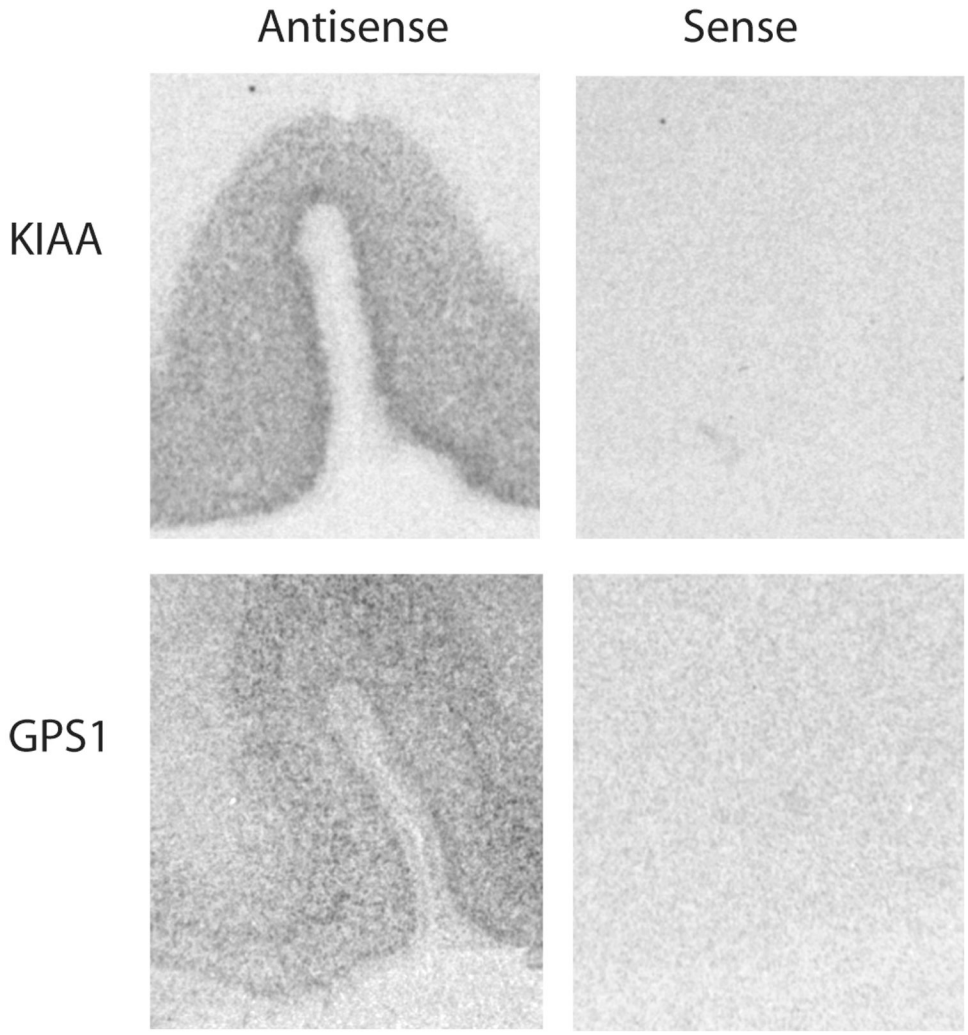


**Figure 3. Probe specificity of KIAA0302 and GPS1**

In situ hybridization analysis of KIAA0302 and GPS1 mRNA expression using sense and antisense probes in anterior cingulate cortex. Abbreviations: KIAA0302 (KIAA).

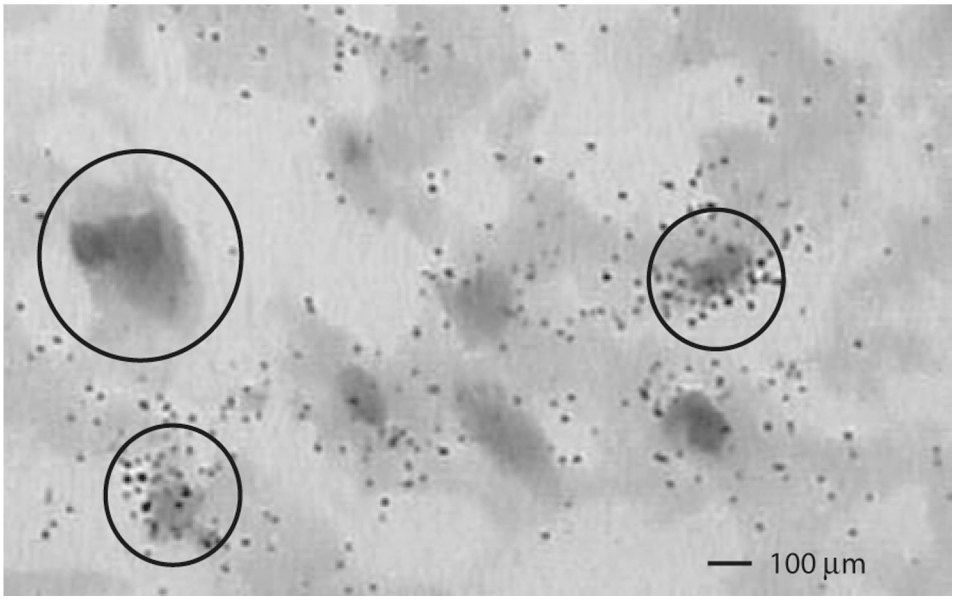


**Figure 4. Cellular expression pattern of EAAT2**

Emulsion dipped in situ hybridization of EAAT2 mRNA in nissl stained tissue from the vicinity of layer V of anterior cingulate cortex. Large circle indicates large cell, small circles indicate small cells.

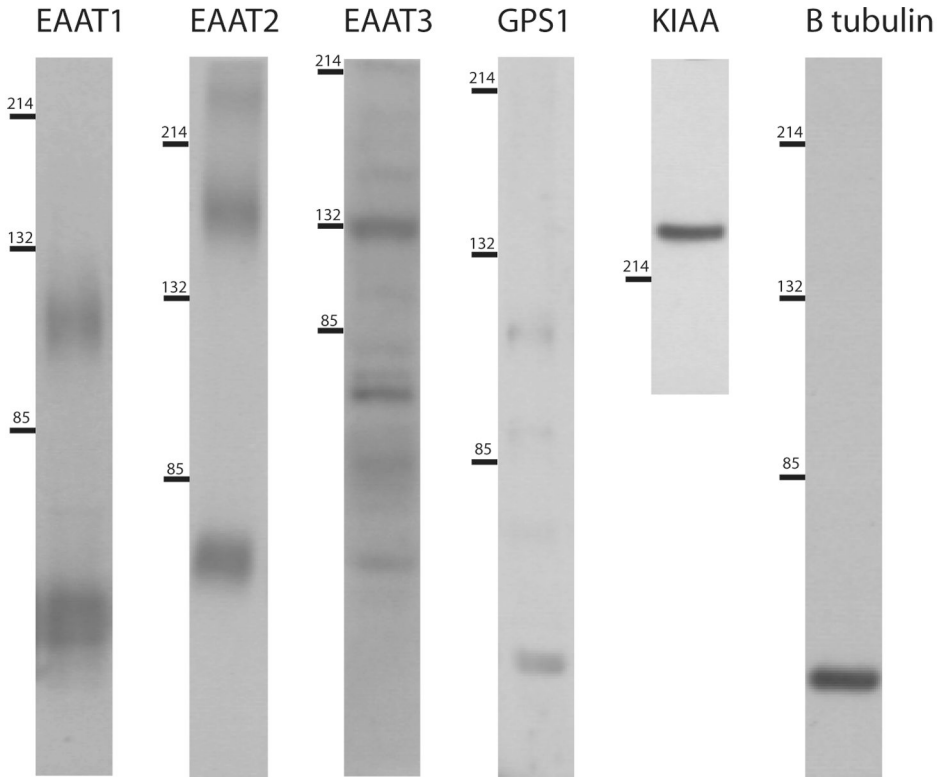


**Figure 5. Protein expression profiles**

Western blot analysis of all proteins studied. Numbers on left indicate molecular weight in kDa. Abbreviations: KIAA0302 (KIAA).

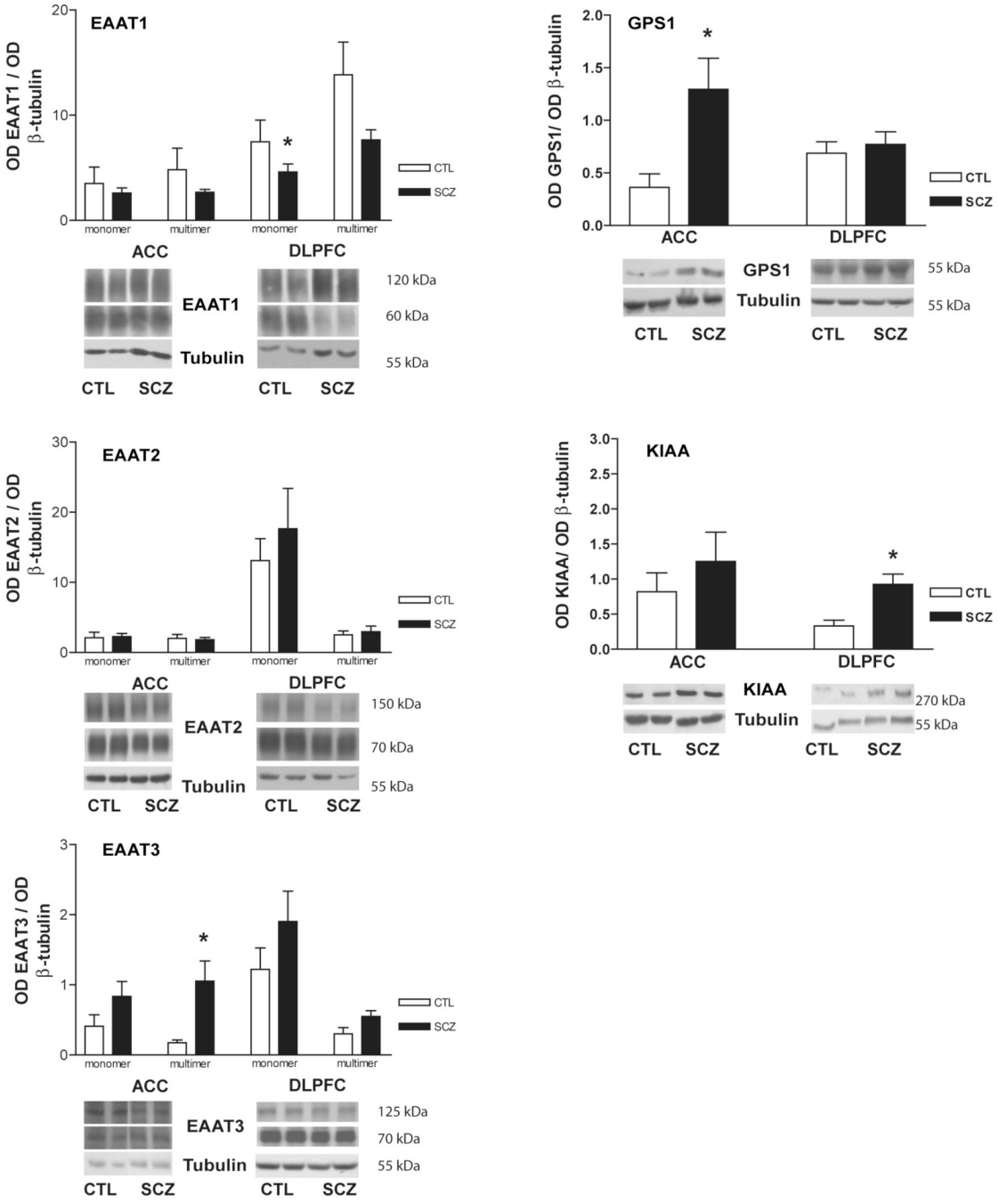


**Figure 6. EAAT and EAAT interacting partner protein expression**

Western blot analysis in the anterior cingulate cortex and dorsolateral prefrontal cortex from control and schizophrenia subjects for EAAT1–3, GPS1, and KIAA0302. Values are normalized to tubulin. Asterisks indicate a significant difference between control and schizophrenia ($p < 0.05$). Data expressed as means +/− standard error of the mean. Abbreviations: Optical density (OD), control (CTL), schizophrenia (SCZ), anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DLPFC), excitatory amino acid transporter (EAAT).

**Table 1**

Subject Characteristics for In Situ Hybridizations

| Age (Years) | Sex | PMI (min) | pH | Cause of Death | | | |
|---|---|---|---|---|---|---|---|
| **Control Subjects** | | | | | | | |
| 79* | F | 181 | 6.3 | CPF | | | |
| 96* | F | 195 | 6.7 | CPF | | | |
| 90* | F | 250 | 6.0 | CPF | | | |
| 69 | M | 255 | 6.3 | Unknown | | | |
| 64 | F | 1145 | 6.1 | Pulmonary edema | | | |
| 93 | M | 1140 | 6.4 | Congestive heart failure | | | |
| 102 | F | 423 | 6.5 | Acute Myocardial Infarction | | | |
| 73 | F | 203 | 6.3 | Acute Myocardial Infarction | | | |
| 79 | F | 460 | 6.5 | Acute Myocardial Infarction | | | |
| 84 | F | 1110 | 6.2 | Unknown | | | |
| 101 | M | 280 | 6.8 | Coronary artery disease | | | |
| mean ± SD: 85 ± 13 | 3M / 8F | 513 ± 407 | 6.4 ± 0.2 | | | | |
| **Subjects with Schizophrenia** | | | | | **Subtype** | **Medications** | **Weeks med free** |
| 84 | F | 935 | 6.2 | Unknown | par | thi/chl | 115 |
| 61* | M | 212 | 6.5 | CPF | undif | per/hal/ser | 1 |
| 69* | M | 270 | 6.4 | Cardiac infarction, renal failure | par | hal/flu/tri/chl | 6 |
| 63* | M | 372 | 5.9 | CPF | undif | hal | 0 |
| 69* | F | 820 | 6.2 | CPF | undiff | thi/tri | 4 |
| 87* | M | 670 | 6.5 | CPF | undif | thi/tri | 11 |
| 68* | M | 335 | 6.8 | CPF | par | pro | 2 |
| 85 | M | 320 | 6.3 | CPF | undiff | flu/chl | 0 |
| 73 | M | 475 | 6.5 | Cardiorespiratory failure | disorg | hal | 0 |
| 66 | M | 725 | 6.5 | Acute cardiac failure | undif | unknown | unknown |
| 76 | F | 1270 | 6.1 | Cardiogenic shock | par | flu/tri/chl | 0 |
| 97 | M | 555 | 6.5 | CPF | undif | hal | 0 |
| 66 | M | 504 | 6.7 | CPF | sa | hal | 0 |
| 82 | F | 1126 | 6.6 | CPF | res | ris | 124 |
| 79 | F | 595 | 6.8 | Cardiac Arrest | undif | thx | 364 |

| Age (Years) | Sex | PMI (min) | pH | Cause of Death | | | |
|---|---|---|---|---|---|---|---|
| 68 | M | 1036 | 6.6 | CPF | cat | flu | 0 |
| 86 | F | 415 | 5.8 | Respiratory insufficiency, renal failure | cat | thx/chl | 36 |
| 65* | F | 350 | 5.9 | CPF | undif | unknown | unknown |
| 79* | F | 1225 | 7.1 | CPF, cancer of pancreas | undif | thi/thx | 9 |
| 84* | M | 372 | 6.5 | CPF | undif | hal/per/thi/chl | 106 |
| mean ± SD: 75 ± 10 | 12M / 8F | 629 ± 333 | 6.5 ± 0.3 | | | | |

Abbreviations: post-mortem interval (PMI), standard deviation (SD), female (F), male (M), cardiopulmonary failure (CPF), paranoid (par), undifferentiated (undif), disorganized (disorg), schizoaffective (sa), residual (res), catatonic (cat), medication (med), Thioridazine (thi), Chlorpromazine (chl), Perphenazine (per), Haloperidol (hal), Trifluoperazine (tri), Prochlorperazine (pro), Fluphenazine (flu), Thiothixene (thx), Risperidone (ris).

Asterisks indicate subject is present in both in situ hybridization and Western blot analysis cohorts.

**Table 2**

Subject Characteristics for Western Blot Analyses

| Age (Years) | Sex | PMI (Min) | pH | Cause of Death | | | |
|---|---|---|---|---|---|---|---|
| **Control Subjects** | | | | | | | |
| 88 | M | 285 | 5.9 | Cardiac | | | |
| 86 | F | 280 | 6.5 | Unknown | | | |
| 55 | M | 600 | 5.7 | Cancer | | | |
| 79* | F | 181 | 6.3 | CPF | | | |
| 96* | F | 195 | 6.7 | CPF | | | |
| 90* | F | 250 | 6.0 | CPF | | | |
| 74 | F | 180 | 6.0 | CPF | | | |
| 70 | M | 482 | 6.0 | | | | |
| mean ± SD: 74 ± 13 | 3M / 5F | 306 ± 154 | 6.1 ± 0.3 | | | | |
| **Subjects with Schizophrenia** | | | | | **Subtype** | **Medications** | **Weeks med free** |
| 54 | M | 490 | 6.0 | Acute myelocytic leukemia | disorg | unknown | unknown |
| 61* | M | 212 | 6.5 | CPF | undif | per/hal/ser | 1 |
| 84* | M | 372 | 6.5 | CPF | undif | hal/per/thi/chl | 106 |
| 69* | M | 270 | 6.4 | Cardiac infarction, renal failure | failure par | hal/flu/tri/chl | 6 |
| 76 | F | 510 | 6.1 | CPF, breast cancer | par | thi | 0 |
| 87* | M | 670 | 6.5 | CPF | undif | thi/tri | 11 |
| 68* | M | 335 | 6.8 | CPF | par | pro | 2 |
| 86 | F | 330 | 6.2 | Cardiac, pneumonia | undif | unknown | unknown |
| 72 | M | 1235 | 6.6 | CPF | undif | neuroleptic | 12 |
| 65* | F | 350 | 5.9 | CPF | undif | unknown | unknown |
| 79* | F | 1225 | 7.1 | CPF, pancreatic cancer | undif | thi/thx | 9 |
| 63* | M | 372 | 5.9 | CPF | undif | hal | 0 |
| 69* | F | 820 | 6.2 | CPF | undif | thi/tri | 4 |
| mean ± SD: 72 ± 13 | 8M / 5F | 553 ± 342 | 6.4 ± 0.4 | | | | |

Abbreviations: post-mortem interval (PMI), standard deviation (SD), female (F), male (M), cardiopulmonary failure (CPF), paranoid (par), undifferentiated (undif), disorganized (disorg), medication (med), Thioridazine (thi), Chlorpromazine (chl), Perphenazine (per), Haloperidol (hal), Fluphenazine (flu), Trifluoperazine (tri), Prochlorperazine (pro), Thiothixene (thx).

Asterisks indicate subject is present in both in situ hybridization and Western blot analysis cohorts.