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DNA fragmentation is increased in non-GABAergic neurons in bipolar disorder but not in schizophrenia

Ned Buttner, Sujoy Bhattacharyya, John Walsh, and Francine M. Benes

Laboratories for Structural Neuroscience, McLean Hospital, Belmont, MA; Program in Neuroscience and Department of Psychiatry, Harvard Medical School, Boston, MA

Abstract

Apoptosis is thought to contribute to neuronal loss in bipolar disorder and schizophrenia, although empiric evidence in support of this idea has been lacking. In this study, we investigated whether or not apoptosis is associated with GABAergic interneurons in the anterior cingulate cortex in schizophrenia (n = 14) and bipolar disorder (n = 14) when compared to normal controls (n = 14). A double-labeling technique using the Klenow method of *in situ* end-labeling (ISEL) of single-stranded DNA breaks was combined with an *in situ* hybridization localization of mRNA for the 67 kiloDalton (kDa) isoform of glutamate decarboxylase (GAD67) and applied to the anterior cingulate cortex of 14 normal controls, 14 schizophrenics, and 14 patients with bipolar disorder matched for age and postmortem interval. An increase in Klenow-positive, GAD67-negative nuclei was observed in layer V/VI of patients with bipolar disorder, but not schizophrenics. Klenow-positive cells that were also positive for GAD67 mRNA did not show differences in either patient group. Conclusions: This is the first demonstration that there is more DNA fragmentation in cells showing no detectable GAD67 mRNA in patients with bipolar disorder than in schizophrenics or controls. These findings suggest that non-GABAergic cells may be selectively vulnerable to oxidative stress in patients with bipolar disorder.

Keywords

schizophrenia; bipolar disorder; apoptosis; GABA; anterior cingulate cortex

1. Introduction

Dysfunction of the anterior cingulate cortex (ACCx) has been suggested to contribute to the pathogenesis of both schizophrenia and bipolar disorder (Benes et al., 1991). Functional imaging experiments show aberrant perfusion and metabolism in this region of both disorders (Baker et al., 1997; Erkwow et al., 1997; Haznedar et al., 1997; Rubinsztein et al., 2001), while magnetic resonance studies demonstrate decreased gray matter volume in patients with bipolar disorder (Drevets et al., 1997; Lopez-Larson et al., 2002). At the histopathological level, a reduction in laminar thickness and neuron densities in layers III, V, and VI of ACCx (Bouras et al., 2001) as well as decreases in glial cell density have been reported in ACCx of bipolars (Knable, 1999; Ongur et al., 1998). Synaptic abnormalities have also been reported in the ACCx of bipolars with reductions in proteins, such as synaptophysin, complexin II, and GAP-43

Corresponding Author Francine M. Benes, MD, PhD, McLean Hospital, 115 Mill Street, Belmont, MA 02478, USA. Telephone: (617) 855-2401, FAX: (617) 855-3199, E-Mail: fbenes@mclean.harvard.edu .

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(Eastwood and Harrison, 2001). Other post-mortem studies have provided evidence for a decrease of GABAergic cells in ACCx and hippocampus in both schizophrenia and bipolar disorder (Benes and Beretta, 2001).

Some believe that neuronal cell death plays a central role in this decrease. Pathological changes in both neurons (Jarskog et al., 2000; Uranova et al., 2001) and glia (Cotter et al., 2001; Knable, 1999; Ongur et al., 1998), similar to those seen in the ACCx of schizophrenics and bipolars, could potentially result from apoptosis or necrosis. A hallmark feature of apoptosis is the presence of DNA breaks, and this is thought to be caused by a fragmentation factor activated by a complex cascade that turns on caspase enzyme (Evan and Littlewood, 1998). This DNA fragmentation factor produces nicks that yield either single-stranded or double-stranded breaks (Ansari et al., 1993). The latter may persist in surviving cells, if they are unable to repair the DNA damage with a polymerase enzyme. Here, we have used the Klenow method of *in-situ* end labeling (ISEL) to co-localize single-stranded DNA breaks (or double-stranded DNA breaks with 5' protruding termini) with an *in situ* hybridization (ISH) of the 67 kDa isoform of glutamate decarboxylase (GAD67), a marker for GABAergic interneurons (Heckers et al., 2002; Jin et al., 1999; Stone et al., 1999). The number of GABAergic and non-GABAergic cells showing DNA fragmentation was assessed to determine whether apoptosis may be involved in neuronal cell loss in schizophrenia and bipolar disorder.

2. Materials and Methods

2.1 Tissue

The cohort was obtained from the Harvard Brain Tissue Resource Center at McLean Hospital and consisted of 14 normal controls, 14 schizophrenics, and 14 patients with bipolar disorder. Cases were matched as precisely as was possible as triplets in terms of age, postmortem interval (PMI), and freezer storage time (Table 1). Three subjects in the schizophrenic group were either neuroleptic-naïve or neuroleptic-free at the time of death. Four subjects in the bipolar disorder group were neuroleptic-naïve or neuroleptic-free. The neuroleptic-free designation was only given to those patients labeled as "0" for CPZ (Table 2).

The presence of senile plaques and neurofibrillary tangles were evaluated in cases obtained prior to 1999 using the CERAD criteria (Davidson et al., 1996) and in those obtained after 1999 using the Braak criteria (Newell et al., 1999). None of the subjects in this study received a neuropathological diagnosis of senile dementia of the Alzheimer's type according to either set of criteria.

Fresh blocks of ACCx (Brodmann area 24) were sectioned on a cryostat at a thickness of 10 μ m and mounted on glass slides as triplets (Benes et al., 1992; Heckers et al., 2002). Two transverse sections of ACCx were analyzed for each case, and layers II and V/VI were analyzed for each section.

2.2 In Situ End-Labeling

Single-stranded DNA breaks were detected with a TACS 1 Klenow Apoptosis Detection Kit (Trevigen, Inc.; Gaithersburg, MD, USA). ISEL processing began with a 10-minute post fixation in 4% paraformaldehyde (in 0.1% diethylpyrocarbonate treated PBS; henceforth DEPC-PBS) at room temperature. Sections were rehydrated for ten minutes in DEPC-PBS, and permeabilized in Cytopore™ (Trevigen, Inc.) for 20 minutes. The sections were quenched of endogenous peroxidase in 2% H₂O₂ for 5 minutes. This was followed by a 5 minute labeling buffer (0.5 M Tris - pH 7.5, 50 mM MgCl₂, 0.6 mM 2-mercaptoethanesulfonic acid, 0.5 mg/ml BSA) wash prior to treatment with the Klenow labeling mixture. The slides were blotted dry and a mixture of the Klenow enzyme and biotinylated dNTPs in labeling buffer was applied

to each section. The slides were then coverslipped and placed in a 37°C incubator for 1 hour. Following the incubation, the sections were placed in a stop buffer (3 M NaCl, 300mM sodium citrate) for five minutes and ISH was immediately begun.

2.3 Riboprobe preparation

The 3 kilobase (kb) GAD67 clone (provided by Drs. A. Tobin and N. Tillakaratne, UCLA) was chosen as a label for GABAergic interneurons. The plasmids were linearized and then incubated for 2 hours at 37°C in a mixture of T7 RNA polymerase (1 µl; Roche Diagnostics), 10 mM NTP (minus UTP) mixture (1 µl; Roche Diagnostics), [³⁵S]-labeled UTPs (250 µCi; New England Nuclear), 5X transcription buffer (2.0 µl), 0.1 M dithiothreitol (1.0 µl), RNasin (0.5 µl; Promega), and DEPC-treated dH₂O (3.5 µl). The DNA template was removed by adding 0.5 µl RQ1 DNase (Promega, Madison, WI, USA) with 6µl 0.1 M DTT and 12 µl 5X transcription buffer in 42 µl DEPC-H₂O and incubating for 15 minutes at 37°C. Unincorporated NTPs and ([³⁵S]-labeled UTPs were removed with a Stratagene (La Jolla, CA, USA) Nucrap purification column. Collected probe was stored at -20°C until hydrolysis. To ensure optimal probe size (0.8 kb) the riboprobe was hydrolyzed in 100 µl of pH 10.2 sodium carbonate buffer (40µl 200mM NaHCO₃ + 60µl 200mM Na₂CO₃) for 5 minutes at 60°C. The hydrolysis reaction was stopped by adding a mixture of 6 µl sodium acetate and 10 µl 10% glacial acetic acid. The probe was precipitated by adding 14 µl of 3 M sodium acetate, 100 µg of yeast tRNA, and 575 µl of ethanol and then centrifuged at 14,000 rpm at 4°C for 20 minutes. The riboprobe pellet was resuspended in hybridization buffer (0.5 M EDTA, 4X SSC, 1X Denhardt's solution, 10 mM dithiothreitol, in 0.02 % sodium dodecylsulfate, 0.1% yeast tRNA, 0.1% ssDNA, 10% dextran sulfate, 50% formamide) to a total volume of 500 µl and stored at -20°C.

2.4 In Situ Hybridization

All sections were acetylated by incubating for 5 minutes in 0.1 M triethanolamine followed by 10 minutes in 0.1M triethanolamine containing 0.1% acetic anhydride. The sections were rinsed twice for 5 minutes in DEPC-PBS and dehydrated in a graded series of ethanol. The sections were placed in a prewarmed, prehumidified chamber containing 50% formamide in DEPC-H₂O, and 20 µl of the riboprobe/hybridization buffer mixture was placed on each section. The sections were coverslipped and placed in a 55°C oven for 3 hours. Following hybridization, the coverslips were removed by soaking in 4X SSC (0.7% 2-mercaptoethanol) and sections were placed in 0.5 M NaCl, 0.05 M PB for 10 minutes. The sections then underwent incubation in 0.5 M NaCl, 0.05 M PB with 0.025 mg/ml RNase A (Sigma) for 30 minutes at 30°C, followed by a high stringency wash for 30 minutes at 63°C in 50% formamide, 0.5 mM NaCl, 0.05 M PB, 0.7% 2-mercaptoethanol (to a total volume of 200 ml). The sections were washed overnight in 0.5X SSC, 20 mM 2-mercaptoethanol at room temperature, and returned to 0.1 M PBS.

2.5 ISEL and ISH Visualization

After completing the ISH processing, the slides were incubated in streptavidin/horseradish peroxidase (Zymed, Inc.) in PBS for 20 minutes. The sections were washed twice for two minutes each in 0.1 M PBS. Single-stranded break-specific signal was developed by submerging the sections in a solution of 0.05% DAB and 0.01% hydrogen peroxide in PBS for 8 minutes. The visualization process was stopped by rinsing briefly in dH₂O. After drying, slides were apposed to x-ray film (Kodak Biomax MS). Sufficient autoradiographic intensity developed after 4 days of exposure. The sections were dipped in autoradiographic emulsion (Kodak NTB2) and left to expose for 1 month. The slides were developed (Kodak D-19 developer), fixed (Kodak fixer), and washed in dH₂O. All sections were immediately counterstained with 0.5% methyl green, dehydrated in graded ethanols, cleared in xylene, mounted with Cytoseal (Fisher Scientific) and coverslipped.

In preliminary experiments, the compatibility of the ISEL localization of single-stranded DNA breaks with ISH localization of GAD67 mRNA was evaluated by performing one or both paradigms in adjacent sections of ACCx and by altering the sequence with which each procedure was performed. When ISEL labeling was compared to GAD67 ISH, a decrease in both labels was observed, whether the ISEL was performed first or second. The signal-to-noise ratio for the ISH procedure was reduced because the specific labeling was diminished and the number of background grains was increased. This pattern occurred whether the ISH was run first or second. If the ISH procedure was run first, the amount of labeling for DNA breaks was significantly increased when compared to the single localization, suggesting that the ISH processing adversely affected double-stranded DNA in the cells. To avoid non-specific ISEL labeling, ISH was performed after the latter had been completed. Autoradiographic grain clusters were not observed in the white matter in any cases.

2.6 Microscopic Analyses

The slides were codified and analyzed under blind conditions using a Leitz Laborlux microscope equipped with a solid-state video camera interfaced with a Bioquant Image Analysis System (R&M Biometrics, Inc., Nashville, TN). Initially, a column of cortex (300 μm wide) was identified under a 4x objective lens. The sampling field extended across the six cortical layers from the pial surface above layer I to the interface of layer VI with the underlying white matter. Klenow staining, GAD67 ISH, and nuclear methyl-green counterstaining were evaluated at low and high power. ISEL-positive cells were identified by the presence of a characteristic brown DAB reaction product appearing as diffuse nuclear staining, chromatin clumps, or nuclear blebs (Benes et al., 2003). The majority of nuclei showed no ISEL-positive staining. Using a 10x objective, the slide was positioned so that layer II was centered and in focus. With the 40x objective lens, the number of Klenow-positive nuclei were touch-counted in the image window. For details of microscopic sampling, see Supplementary Materials.

2.7 Statistical Analyses

When the code was broken, the data for layers II and V/VI of each case were compiled and an average $\pm$ standard deviation was determined for the Klenow-positive cells that were either GAD67-positive or GAD67-negative. An analysis of variance (ANOVA) was completed on each layer to determine whether the differences between the groups were significant. Simple pair-wise correlation coefficients were obtained to evaluate whether there were any significant relationships between the labeled cells and the confounding variables. Analysis of covariance (ANCOVA) was then used to evaluate whether any of these relationships might have influenced the results. Multiple regression analyses were performed to rule out the effect of manifold confounding variables.

3. Results

Klenow-positive neurons were detected by the presence of a brown precipitate in and around the nucleus (Fig. 1). Because of this relatively light cellular DAB staining and the superimposition of autoradiographic emulsion, fine morphological details of nuclei were not distinguishable. Specifically, subtle morphological features of nuclei with diffuse, light staining or those showing Klenow-positive clumps or blebbing, as previously described (Benes et al., 2003), could not be consistently discerned. Thus we did not observe or quantitate a gradation of Klenow-positive staining but rather characterized cells simply as either Klenow-positive or Klenow-negative based on the presence or absence of distinct brown staining within the cellular profile. Neuronal nuclei were differentiated from non-neuronal nuclei by their scarcity in the subcortical white matter and by their size, which is larger than that of typical glia. GAD67 autoradiographic signal was uniform across all slides and no differences were observed in the non-specific background signal between the three diagnostic groups.

As shown in Fig. 2, the grain densities for all Klenow-positive cells, both GAD67-positive and GAD67-negative, did not show significant differences between the three groups. It was possible, however, using the specific density of GAD67 autoradiographic grains covering Klenow-positive cells, to discriminate two separate groups that were either positive or negative for GAD67 based on whether or not they exceeded 2X background signal (Fig. 1a). When Klenow-positive neuronal profiles were grouped according to this criterion, a marked and highly significant difference was observed between cells considered either GAD67-positive or GAD67-negative ($p < 0.0001$, Fig. 2).

There was a significant increase (59%) in the numerical density of Klenow-positive, GAD67-negative neurons in layer V/VI of patients with bipolar disorder versus normal controls ($p < 0.05$) (Figs. 1b and 3). No difference was seen in the number of Klenow-positive, GAD67-positive neurons in layer V/VI between the three groups (Fig. 3). Similarly, no differences were seen in the number of Klenow-positive GAD67-negative neurons or Klenow-positive GAD67-positive neurons in layer II between the normal controls and the two patient groups (Fig. 3). ANCOVA revealed a gender-diagnosis interaction across the groups; however the means for males and females in the bipolar group were the same. It is also possible that suicide is a key variable, but suicide-free bipolar cohorts are difficult to obtain.

As noted above, the three groups were well matched for age, postmortem interval, tissue pH, and neuroleptic exposure (Table 2). No consistent trends were noted in the correlation coefficients for the numerical density of neurons in either layer, nor did ANCOVA reveal significant influences of these variables on the numbers of Klenow-positive, GAD67-negative neurons in layer V/VI in the three diagnoses. Likewise, stepwise incorporation of these covariates into a multiple regression model failed to explain the variance observed in Klenow-positive, GAD67-negative neurons in layer V/VI of patients with bipolar disorder. Therefore, the significant increase in these latter cells in bipolar disorder does not appear to be secondary to these confounding variables. Additionally, the current results do not appear to be related to the presence of Alzheimer's-associated pathology in the three groups, as this possibility was ruled out by detailed neuropathological examination using accepted criteria.

3. Discussion

To our knowledge, this is the first report suggesting that there may be a selective increase of apoptotic activity in layer V/VI ACCx in bipolar disorder when compared to normals or schizophrenics. The DNA damage may not necessarily progress to apoptosis, but it is certainly possible that at least some GABAergic cells damaged in this way might not have survived in either the bipolar or schizophrenic groups. Consistent with the current findings, a recent study has demonstrated that multiple genes associated with the apoptosis cascade are upregulated in bipolar disorder patients (Benes et al., 2006). The results reported here suggest that cells not showing detectable mRNA for GAD67 are selectively vulnerable to apoptosis; however, this latter population may also contain non-pyramidal cells, in which GAD67 mRNA fell below the level of detection with the double-labeling technique employed here. This seems unlikely, however, because the variances shown in Figs. 1 and 2 are well within reasonable limits and do not appear to have been confounded by a selective decrease of GAD67 mRNA expression in GAD67-negative cells.

In an earlier ISEL study (Benes et al., 2003) in which GAD67 was not co-localized, a marked reduction in Klenow-positive neurons was observed in schizophrenics, but not in patients with bipolar disorder. In this latter study, nuclei were categorized as showing Klenow-positive diffuse staining, clumps, or blebs, but such structures could not be resolved consistently under the stringent conditions used for the co-localization of GAD67 mRNA in the current report. This more complex methodology might have altered the nature and distribution of damaged

DNA, making it impossible to show a reduction of Klenow-positive blebs in the schizophrenics. In the earlier study (Benes et al., 2003) it was not possible to distinguish pyramidal and non-pyramidal neurons. Overall, the current results suggest that DNA damage may be a feature of altered function of pyramidal neurons of bipolars, suggesting that the current study has detected changes of a greater magnitude in a cell population where DNA damage may be a stable finding. Analysis of gene expression microarrays of post-mortem hippocampal extracts indicated an upregulation of proapoptosis genes and a downregulation of antioxidation-related genes in bipolar disorder subjects. In schizophrenia subjects, the apoptosis genes are downregulated but the antioxidant genes are expressed at levels comparable to control subjects.

The population of cells showing increased DNA fragmentation may be one that is particularly vulnerable to excitotoxicity (Didier et al., 1996). Postmortem studies have suggested that GABA cells in the ACCx of bipolar subjects may fall victim to oxidative stress (Benes et al., 1992; Benes et al., 2000) that occurs in response to excessive amygdalar stimulation (Benes and Beretta, 2001). On the other hand, some studies have paradoxically suggested that GABAergic cells in hippocampus may be resistant to kainic acid - induced excitotoxicity (Davenport et al., 1990), although conflicting results have been reported with less localized applications of kainic acid (Morin et al., 1998). Excessive excitatory activity in non-GABAergic cells could result in consequent changes in oxidative enzymes associated with mitochondria (Benes, 2000; Coyle and Puttfarcken, 1993). Apoptosis can potentially be triggered through several different metabolic and signaling pathways that involve mitochondrial membrane permeabilization (Debatin et al., 2002).

Recent studies have shown that antidepressants and mood stabilizers regulate a variety of genes involved in cell survival and cell death, such as CREB, BDNF, Bcl-2, MAP kinases, and GSK-3beta (Manji and Duman, 2001; Lenox and Wang, 2003). Lithium carbonate, the major drug used to treat bipolar disorder, protects cultured rat brain neurons from NMDA-mediated excitotoxicity via mechanisms that include increased expression of Bcl-2 (Chuang et al., 2002). Chronic lithium treatment increases the levels of Bcl-2 in rat frontal cortex, hippocampus, and striatum as well as in cells of human neuronal origin (Manji et al., 2000). Valproate, a mood stabilizing agent for patients with bipolar disorder, also increases expression of Bcl-2 in rat cerebral cortex (Wang et al., 2003), whereas lithium has been shown to inhibit GSK-3beta, a proapoptotic enzyme that inhibits the activation of several cell survival factors such as heat shock factor 1 (Li et al., 2002). Consistent with these changes in Bcl-2 and GSK-3beta, lithium has been found to exert protective effects against cellular insults both in vivo and in vitro (Manji et al., 2000). These recent findings raise the possibility that lithium carbonate and mood stabilizing anticonvulsants might exert some of their therapeutic actions via neuroprotective effects. The fact that a high proportion of bipolar subjects included in this study were treated with lithium and/or anticonvulsants (see Table 1) suggests that apoptotic changes could potentially have been much greater if these drugs had not been prescribed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

(a) GAD67 grain density was determined automatically for all Klenow-positive nuclei. Nuclei exceeding 2X background grain density were also considered GAD67-positive, i.e., double-labeled (white arrow). The cell to the left (black arrow) did not exceed our 2X criterion and was considered Klenow-positive only. (b) An increase in Klenow-positive, GAD67-negative cells (black arrows) was observed in patients with bipolar disorder. The cell to the right in plate B was considered Klenow-negative, GAD67-positive and is included as a comparison (red arrow). Image magnification is 40X.

Fig. 2.

A set of bar graphs showing the mean densities (grains/ μm^2 , $\pm$ SEM) of autoradiographic grains for Klenow-positive, GAD-positive and Klenow-positive, GAD-negative nuclei in normal controls (CON), schizophrenics (SZ), and patients with bipolar disorder (BD). No significant differences were found between diagnoses within cell types (Klenow+/GAD+ or Klenow+/GAD-) in either region. Differences in grain densities, however, between Klenow+/GAD+ cells and Klenow+/GAD- cells were highly significant in both cortical regions (***) $p < 0.0001$.

Fig. 3.

A set of bar graphs showing the mean ($\pm$ SEM) for Klenow-positive nuclei counted per objective field in normal controls (CON), schizophrenics (SZ) and patients with bipolar disorder (BD). The mean is increased by 59% in GAD-negative nuclei in layer V/VI of patients with bipolar disorder when compared to normal controls. The asterisk indicates that the difference in the bipolar disorder group is significant at the $p < 0.05$ level.

Table 1
Mean and standard deviation of associated demographic data

Diagnosis	N	Age, yr	PMI, hr	Sex, M/F	Hemisphere, L/R	Tissue pH	Freezer storage, days	Neuroleptic Medication
Normal Control	14	61 ± 18.8	18.5 ± 7.2	8/6	6/8	6.51 ± 0.23	1457 ± 288	-
Schizophrenic	14	60.8 ± 19.4	20.7 ± 7.0	7/7	4/8	6.37 ± 0.36	2010 ± 237	482 ± 292
Bipolar	14	60.3 ± 18.6	18.0 ± 7.7	9/5	7/5	6.49 ± 0.26	1979 ± 344	344 ± 161

Neuroleptic medication for one year prior to death is expressed as CPZ-equivalents, mg.

Table 2

Demographic data by case

Diagnosis and brain number	Age, yr	PMI, hr	Sex	pH	Mood Stabilizer	CPZ-equiv	Hemisphere, L or R	Cause of Death
CON 1	75	5.5	M	n/avail.		.	L	Cardiopulmonary Arrest
CON 2	66	18.7	M	6.76		.	R	Myocardial Infarction
CON 3	44	23	M	6.71		.	R	Cardiopulmonary Arrest
CON 4	22	24.3	M	6.68		.	R	Motor Vehicle Accident
CON 5	84	11.8	F	6.35		.	L	Cancer
CON 6	78	14.1	F	6.22		.	R	Myocardial Infarction
CON 7	78	14.1	F	6.51		.	R	Aortic Aneurysm
CON 8	38	21.9	M	6.63		.	L	Myocardial Infarction
CON 9	44	10.7	F	6.49		.	L	Myocardial Infarction
CON 10	52	32.1	M	6.53		.	L	Cardiopulmonary Arrest
CON 11	54	24.2	M	6.42		.	L	Cardiopulmonary Arrest
CON 12	67	22.3	M	6.74		.	R	Pancreatic Cancer
CON 13	74	12.1	F	6.25		.	R	Cancer
CON 14	78	23.9	F	6.03		.	R	Cancer
SZ 1	33	28	M	n/avail.		3000	L	Stab Wound to Neck
SZ 2	43	8.8	M	n/avail.		400	L	Myocardial Infarction
SZ 3	78	6.5	F	n/avail.		100	L	Renal Failure
SZ 4	89	13.5	F	n/avail.		20	L	Pneumonia
SZ 5	43	26	F	n/avail.		750	L	Burns
SZ 6	82	20	F	6.08		0	R	Myocardial Infarction
SZ 7	66	22.1	M	6.43		na	R	Emphysema
SZ 8	46	29.3	M	7.1		na	R	Cancer
SZ 9	83	23.2	F	5.91		0	R	Cardiopulmonary Arrest
SZ 10	84	25.7	F	6.14		50	R	Cardiopulmonary Arrest
SZ 11	44	19	M	6.2		na	L	Pneumonia
SZ 12	42	27.1	M	6.64		0	R	Cancer
SZ 13	55	18	F	6.52		na	R	Cancer
SZ 14	63	22.3	M	6.55		500	R	Myocardial Infarction
BD 1	65	4.5	F	n/avail.	LiCO ₃	500	R	Pulmonary Disease
BD 2	42	13	M	n/avail.	0	0	L	Suicide by Suffocation
BD 3	46	15	M	n/avail.	na	1200	L	Myocardial Infarction
BD 4	76	11.5	M	n/avail.	methylphenidate	0	L	Cancer
BD 5	39	21	M	n/avail.	divalproex	250	L	Myocardial Infarction
BD 6	24	24.7	M	6.65	LiCO ₃ , divalproex	0	L	Suicide by Hanging
BD 7	91	13	F	6.19	na	na	R	Pneumonia
BD 8	51	31	M	7.02	Clonazepam, gabapentin	na	L	Suicide by Overdose
BD 9	64	11	F	6.69	na	800	R	Respiratory Failure
BD 10	50	30.5	M	6.51	na	na	R	Cardiac Arrest
BD 11	74	24.8	M	6.35	divalproex	na	L	Pneumonia
BD 12	75	20.5	F	6.53	0	0	L	Myocardial Infarction
BD 13	74	14.3	M	6.32	LiCO ₃ , divalproex, Carbamazepine	na	R	Pneumonia
BD 14	73	17	F	6.27	divalproex	na	R	Renal Failure

Diagnosis codes: CON, control; SZ, schizophrenic; BD, bipolar disorder. PMI, post-mortem interval. Hemisphere (Left or Right) source of brain tissue; CPZ, dose of neuroleptic drugs, expressed in CPZ equivalents.