

# Human Dysbindin (*DTNBP1*) Gene Expression in Normal Brain and in Schizophrenic Prefrontal Cortex and Midbrain

Cynthia Shannon Weickert, PhD; Richard E. Straub, PhD; Benjamin W. McClintock, BA; Mitsuyuki Matsumoto, PhD; Ryota Hashimoto, MD, PhD; Thomas M. Hyde, MD, PhD; Mary M. Herman, MD; Daniel R. Weinberger, MD; Joel E. Kleinman, MD, PhD

**Context:** The schizophrenia-susceptibility gene dysbindin (*DTNBP1* on 6p22.3) encodes a neuronal protein that binds to  $\beta$ -dystrobrevin and may be part of the dystrophin protein complex. Little is known about dysbindin expression in normal or schizophrenic brain.

**Objectives:** To determine whether brain regions implicated in schizophrenia express dysbindin and whether abnormal levels of dysbindin messenger RNA (mRNA) may be found in this disorder and to test whether sequence variations in the dysbindin gene in the promoter region, 5' and 3' untranslated regions, or introns would affect dysbindin mRNA levels.

**Methods:** In patients with schizophrenia and controls, we compared dysbindin, synaptophysin, spinophilin, and cyclophilin mRNA levels in the dorsolateral prefrontal cortex and dysbindin mRNA levels in the midbrain by in situ hybridization. We genotyped brain DNA at 11 single nucleotide polymorphisms to determine whether genetic variation in the dysbindin gene affects cortical dysbindin mRNA levels.

**Main Outcome Measures:** Quantitative assessment of dysbindin mRNA levels across various brain regions

and comparative studies of dysbindin mRNA levels in brains of patients with schizophrenia compared with normal controls.

**Results:** Dysbindin mRNA was detected in the frontal cortex, temporal cortex, hippocampus, caudate, putamen, nucleus accumbens, amygdala, thalamus, and midbrain of the adult brain. Patients with schizophrenia had statistically significantly reduced dysbindin mRNA levels in multiple layers of the dorsolateral prefrontal cortex, whereas synaptophysin, spinophilin, and cyclophilin mRNA levels were unchanged. Dysbindin mRNA levels were quantitatively reduced in the midbrain of patients with schizophrenia, but not statistically significantly. Cortical dysbindin mRNA levels varied statistically significantly according to dysbindin genotype.

**Conclusions:** Dysbindin mRNA is expressed widely in the brain, and its expression is reduced in schizophrenia. Variation in dysbindin mRNA levels may be determined in part by variation in the promoter and the 5' and 3' untranslated regions. These data add to the evidence that dysbindin is an etiologic factor in schizophrenia risk.

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From the Clinical Brain Disorders Branch, Intramural Research Program, National Institute of Mental Health, National Institutes of Health, Bethesda, Md (Drs Weickert, Straub, Hyde, Herman, Weinberger, and Kleinman, and Mr McClintock); Department of Genomics Research, Yamanouchi Pharmaceutical Co, Ibaraki, Japan (Dr Matsumoto); and Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan (Dr Hashimoto).

**I**NHERITANCE OF CERTAIN FORMS OF genes can increase the risk of developing schizophrenia<sup>1,2</sup>; however, it is unclear how these “vulnerability” alleles contribute to aberrant functioning of the brain and lead to the symptoms associated with schizophrenia.<sup>3</sup> One possibility is that alleles that increase risk for schizophrenia may directly affect the development, maturation, and adult function of the dorsolateral prefrontal cortex (DLPFC),<sup>4</sup> an area where cellular and molecular abnormalities are found in the schizophrenic brain.<sup>5-9</sup> Recently, alleles of the gene that encodes dysbindin ( $\beta$ -dystrobrevin binding pro-

tein or *DTNBP1* on 6p22.3), whose protein product has been localized to neurons in the central nervous system,<sup>10,11</sup> have been shown to increase the risk of developing schizophrenia.<sup>12-14</sup> However, to our knowledge, little information is currently available on the brain regions that contain dysbindin messenger RNA (mRNA) or protein in either healthy or psychiatrically compromised human brain. This lack of information limits our ability to delineate anatomic brain areas and molecular pathways that may be affected by the inheritance of dysbindin risk alleles in schizophrenia. One of the central challenges in the genetics of complex hu-

man traits, such as schizophrenia, is uncovering how cis-acting polymorphisms in a susceptibility gene may lead to altered gene regulation.<sup>15</sup> Therefore, in this study, we also examine whether known single nucleotide polymorphisms (SNPs) affect dysbindin mRNA levels.

Dysbindin is a 40- to 50-kDa protein that is ubiquitously expressed in rodent tissues and that binds to  $\alpha$ - and  $\beta$ -dystrobrevin in muscle and brain, respectively.<sup>11</sup> The dystrobrevins associate with dystrophin, the product of the gene mutated in Duchenne muscular dystrophy, which, together with syntrophins and dystroglycan, form the core of the dystrophin protein complex (DPC) (for a review, see Watkins et al<sup>16</sup>). The DPC has been studied primarily in muscle, where it stabilizes the postsynaptic membrane, is involved in cytoskeletal rearrangement, and perhaps facilitates the transduction of extracellular signals.<sup>17,18</sup> Because patients with Duchenne muscular dystrophy have cognitive dysfunction and occasionally mental retardation,<sup>19</sup> it has been proposed that the DPC may be involved in the maintenance of structure and function of the postsynaptic membranes of central nervous system neurons.<sup>20</sup> Components of the DPC that have been localized to cortical neurons and more specifically to neuronal postsynaptic densities include dystrophin, syntrophin, and dystrobrevin.<sup>19,21,22</sup> Dysbindin protein has also been localized to postsynaptic sites, particularly in Purkinje cell soma and dendrites in the mouse cerebellum.<sup>10</sup> Somal dysbindin may play a role in organelle biogenesis by binding to proteins such as pallidin and muted, which are involved in organelle trafficking.<sup>23</sup> However, another established binding partner for dysbindin,  $\beta$ -dystrobrevin, has been associated with presynaptic terminals in photoreceptors in the avian retina, implying that dysbindin may also be localized in axon terminals.<sup>24</sup> Available neuroanatomic evidence<sup>10,11</sup> is consistent with a synaptic localization for dysbindin, as mossy fiber terminal fields in the hippocampus and cerebellum and neuropil areas of the cortex and substantia nigra contain dysbindin protein in the rodent brain. Thus, dysbindin may be located presynaptically and postsynaptically in the central nervous system.

In this study, we characterized the anatomic distribution of dysbindin mRNA in 12 distinct areas of the human brain, with special emphasis on areas most often implicated in schizophrenia.<sup>25</sup> We then tested whether dysbindin expression was altered in the DLPFC and midbrain of patients with schizophrenia. In brain imaging studies and cognitive assessments, the DLPFC has consistently been found to be dysfunctional in patients with schizophrenia.<sup>26,27</sup> Underlying these behavioral and functional deficits, molecular and cellular abnormalities exist, including reduced neuronal size, synaptic connectivity, and plasticity of cortical neurons.<sup>28-37</sup> Given the evidence of a generalized "synaptic pathology" in the brains of patients with schizophrenia, we hypothesized that a reduction in dysbindin mRNA levels might be predicted owing to the presence of fewer presynaptic and postsynaptic sites (where dysbindin can be located). In this scenario, a decrease in dysbindin expression could simply be secondary to changes in synaptic structure. To monitor the magnitude of synaptic change, we also measured the expression of synaptic-associated gene prod-

ucts used as proxies for the number of synaptic terminals. We hypothesized that levels of synaptophysin and spinophilin mRNA, markers of the presynaptic and postsynaptic terminals, respectively, may be reduced in the DLPFC in patients with schizophrenia and that these changes may correlate with changes in dysbindin mRNA levels.

## METHODS

### BRAIN COLLECTION AND ANATOMY

For the normal anatomic distribution studies of dysbindin mRNA, 5 to 7 healthy individuals were used (2 sections per case, a subset of the controls given in **Table 1**). Postmortem brains were obtained as previously described elsewhere.<sup>28,38</sup> For cryostat sectioning of the normal brain, tissue blocks were dissected from the following regions: (1) the DLPFC, blocked at the middle third of the middle frontal gyrus anterior to the corpus callosum; (2) the basal ganglia, blocked at the level of the nucleus accumbens; (3) the midbrain, blocked at the exit of the oculomotor nerve; (4) the rostral mesial temporal lobe, blocked at the amygdala/rostral hippocampus; and (5) the more caudal mesial temporal lobe, blocked at the genu to midbody level of the hippocampus. Eight sections from the postmortem DLPFC of each subject (14 schizophrenic patients and 15 controls [detailed demographics are available in Table 1 and were previously published<sup>29</sup>]) were used in this study (2 sections per case per probe). Sections from the midbrain at the level of the red nucleus were also used to compare dysbindin expression in schizophrenic patients (n=7) with that in normal brain (n=13).

### DIAGNOSIS AND EXPERIMENTAL DESIGN

Patients with schizophrenia and controls were matched for age, tissue pH (determined for each case as previously described elsewhere<sup>28</sup>), postmortem interval (PMI) (defined as the time between death and brain freezing), sex, race, and brain hemisphere. Diagnosis was determined by independent review of clinical records by 2 board-certified psychiatrists who used the *Diagnostic Evaluation After Death (DEAD)*<sup>39,40</sup> as a guide to review the material available on each case as described.<sup>28</sup> Only cases that met DSM-IV criteria for schizophrenia were included, with 3 patients of the chronic undifferentiated subtype, 9 of the chronic disorganized subtype, and 2 of the chronic paranoid subtype. The mean  $\pm$  SD age at disease onset was  $23 \pm 7$  years. All patients diagnosed as having schizophrenia had a history of auditory hallucinations and paranoid delusions. IQ data were available for approximately half of the patients with schizophrenia (full-scale IQ score, mean  $\pm$  SD,  $76 \pm 14$ ). The total dose of neuroleptic medication given to the patients was calculated as described in a previous publication,<sup>28</sup> and detailed clinical information on these schizophrenia cases can be found in a previous publication from our group.<sup>29</sup>

### GENERATION OF RIBOPROBE TEMPLATES

T7/T3 promoter-tagged riboprobe templates were generated using reverse transcription polymerase chain reaction. To avoid nonspecific amplification, a nested amplification scheme was used. We amplified a 290-base pair (bp) template from exons 6, 7, and 8 (positions 490-779, GenBank accession No. BC011912). Under the polymerase chain reaction conditions described in a previous publication,<sup>41</sup> a single dysbindin complementary DNA (cDNA) product of expected size (412 bp) was amplified using the primers 5'-GAGGCGAGTTTGTAGGAGGT-3' (sense)

**Table 1. Characteristics of the Study Cohort**

Brain No.	Diagnosis	Age, y	Sex	Race	Brain Hemisphere	pH	PMI, h	Cause of Death	Manner of Death	Toxicologic Findings
1	CON	52	F	AA	R	6.87	10.0	Hemopericardium	Natural	Opiates in occipital lobe
2	CON	35	M	AA	R	5.88	49.5	ASCVD	Natural	Negative
3	CON	41	M	AA	R	6.72	10.0	Stab wounds to chest	Homicide	Alcohol
4	CON	57	F	AA	R	6.43	19.0	ASCVD	Natural	Negative
5	CON	66	F	W	R	6.37	29.5	Ruptured thoracic aneurysm	Natural	Negative
6	CON	32	M	AA	R	6.77	15.5	GSW to chest	Homicide	Negative
7	CON	24	M	AA	R	6.59	12.5	Fibrinous pericarditis	Natural	Meperidine hydrochloride in blood
8	CON	38	M	AA	R	6.14	32.5	PE	Accidental	Negative
9	CON	18	M	AA	R	6.51	14.5	GSW to back	Homicide	Negative
10	CON	83	M	AA	L	6.01	66.5	Pulmonary artery thrombosis	Natural	Negative
11	CON	56	M	AA	R	6.09	33.0	PE	Natural	Negative
12	CON	63	M	W	L	6.54	19.0	ASCVD	Natural	Negative
13	CON	52	F	AA	L	6.38	26.0	Acute fibrinous pericarditis	Natural	Lidocaine detected in occipital lobe
14	CON	59	F	AA	R	6.57	37.0	Cirrhosis	Natural	Negative
15	CON	67	F	AA	L	6.69	34.0	Pulmonary edema	Natural	Blood alcohol, 28 mg/dL
16	CDS	75	M	AA	L	6.29	41.5	Pending	Natural	Negative
17	CDS	67	F	AA	R	6.63	38.5	Bronchial asthma, COPD	Natural	Negative
18	CDS	31	M	W	R	6.46	14.0	Cerebral edema	Natural	Negative
19	CUS	23	M	AA	L	6.48	42.5	Anoxia due to seizure, no history of seizures	Natural	Negative
20	CPS	60	F	AA	L	6.38	19.0	HCVD	Natural	Amantadine hydrochloride "detected" in brain
21	CUS	30	M	AA	L	6.32	72.5	Pneumonia	Natural	Negative
22	CDS	35	M	AA	L	6.70	79.0	Acute pulmonary embolus	Natural	Negative
23	CPS	80	M	W	L	6.05	13.5	GI bleed	Natural	NA
24	CDS	81	F	W	R	6.78	11.0	ASCVD	Natural	Amantadine hydrochloride "detected" in brain, blood negative
25	CUS	61	F	AA	R	6.74	20.0	Asphyxiation, food bolus	Accidental	Negative
26	CDS	38	M	AA	R	6.50	61.0	Ruptured intestine, acute peritonitis	Natural	Chlorpromazine "detected" in brain
27	CDS	44	M	AA	R	6.28	37.0	Pulmonary abscess, empyema	Natural	Negative
28	CDS	41	F	AA	R	6.08	51.0	ASCVD	Natural	NA
29	CDS	41	M	AA	L	6.63	32.0	ASCVD	Natural	Negative

Abbreviations: AA, African American; ASCVD, arteriosclerotic cardiovascular disease; CDS, chronic disorganized schizophrenia; CON, control; COPD, chronic obstructive pulmonary disease; CPS, chronic paranoid schizophrenia; CUS, chronic undifferentiated schizophrenia; GI, gastrointestinal; GSW, gunshot wound; HCVD, hypertensive cardiovascular disease; L, left; NA, not available; PE, pulmonary embolism; PMI, postmortem interval; R, right; W, white.

and 5'-CAGAGTTCAGGAAGACGTCCA-3' (antisense) from human brain cDNA.<sup>41</sup> We then used the first cDNA fragment as a template and T7/T3 promoter-tagged primers 5'-CAGAGATGCATAATACGACTCACTATAGGGAGAAGGAGGTAGAGAACAACCTGC-3' (sense, artificial T7 promoter) and 5'-CCAAGCCTTCATTAACCCTCACTAAAGGAGATCGCCGCTCTGCAATCTGCA-3' (antisense, artificial T3 promoter). A single band of expected size (355 bp, including promoters) was amplified and sequenced (identical to GenBank accession No. BC011912). Similarly, a single spinophilin/neurabin II product of expected size (608 bp) was amplified using the outer primers 5'-CCTGGAGAATGGCAGCAC-3' (978-994 bp, forward) and 5'-CGGTCTTGACGAA GATACCC-3' (1566-1585 bp, reverse). In the next round of amplification, we used the first cDNA fragment as a template and T7/T3 promoter-tagged primers 5'-CAGAGATGCA TAATACGACTCACTATAGGGAGAGGTAGATGAATCCAAGAAGGA-3' (sense, artificial T7 promoter) and 5'-CCAAGCCTTCATTAACCCTCACTAAAGGGAGACAGGGAACAGCTCCAACCT-3' (antisense, artificial T3 promoter). A single

band of expected size (421 bp, including promoters) was amplified and sequenced (identical to GenBank accession No. AJ401189, positions 1128-1483). We subcloned human synaptophysin cDNA in the pCR2.1 (InVitrogen Corp, Carlsbad, Calif) vector in the sense and antisense directions from a human synaptophysin cDNA clone (provided by Thomas C. Sudhof, MD, Howard Hughes Medical Institute, University of Texas, Southwestern Medical Center, Dallas).<sup>42,43</sup> Our insert spans 591 to 1084 bp of GenBank accession No. X06389 Y00507 and was sequenced for confirmation. The cyclophilin template used was purchased from Ambion Inc, Austin, Tex.

**IN VITRO TRANSCRIPTION OF RIBOPROBES**

Sense and antisense riboprobes for dysbindin, spinophilin, synaptophysin, and cyclophilin were generated from templates using a T7 or T3 polymerase and an in vitro transcription kit as recommended by the manufacturer (Promega Corp, Madison, Wis). The <sup>32</sup>P-UTP (uridine triphosphate) antisense riboprobes (Northern blotting) and <sup>35</sup>S-UTP antisense and sense

**Table 2. Effect of Dysbindin Genotype at 11 SNPs on Cortical Dysbindin mRNA Levels**

Straub et al, <sup>12</sup> 2002	Laboratory No.	Intermarker Distance	Distance From P3230	SNP Identification No.	SNP	Minor Allele Frequency*	SNP Location†	Chromosome 6 Position‡	Valid N§			P Value	
									1/1	2/2 and U	Z¶		
P1320	P3230	NA	0	rs1047631	A/G	0.18	Exon 10 (3' UTR)	15585640	12	10	30	-1.98	.048
	P3236	63031	63031	NA	T/C	0.11	Intron 7	15648671	17	4	33	0.09	.93
	P3170	29505	92536	rs3829893	C/T	0.17	Intron 5	15678176	18	4	35	0.09	.93
	P2381	11916	104452	NA	G/C	0.13	Intron 4	15690092	18	4	35	0.09	.93
	P4078	5188	109640	rs2619545	A/G	0.22	Intron 4	15695280	6	15	45	0	>.99
	P2555	7540	117180	rs2743864	G/A	0.11	Intron 3	15702820	14	6	17	-2.06	.04
	P2215	10851	128031	rs760761	C/T	0.31	Intron 3	15713671	6	13	37	0.18	.86
	P3521	11986	140017	hCV3114520	A/G	0.22	Exon 1 (5' UTR)	15725657	18	4	2	2.89	.004
	P3587	1295	141312	hCV3114519	T/C	0.19	5' Flanking region	15726952	5	15	15	-1.96	.05
	P4211	796	142108	rs2619538	A/T	0.43	5' Flanking region	15727748	8	13	47	-0.36	.72
	P3593	4089	146197	rs742206	A/G	0.15	5' Flanking region	15731837	9	11	25	-1.86	.06

Abbreviations: mRNA, messenger RNA; NA, not applicable; SNP, single nucleotide polymorphism.

\*Based on sample of white individuals.

†Relative to the structure of the transcript BC011912.

‡From the April 2003 freeze at the University of California at Santa Cruz.

§1 Indicates common allele; 2, minor allele.

||Computed statistic for the robust rank-order test.

¶Normal distribution variant value for the *U* score.

riboprobes (for in situ hybridizations) were labeled to a specific activity of 1 to  $2 \times 10^9$  cpm/ $\mu$ g by addition of radiolabeled UTP and were purified by ethanol precipitation.

#### NORTHERN BLOTTING

Northern blotting was performed as previously described elsewhere.<sup>28</sup> Multiple tissue blots (Clontech, Palo Alto, Calif) containing polyA+ RNA from several brain regions of adults (amygdala, caudate, corpus callosum, hippocampus, whole brain, and thalamus) were used to verify the specificity of our dysbindin and spinophilin riboprobes; for the synaptophysin riboprobe, we used Northern blot, normalized by amount of mRNA (catalog No. N3234410; BioChain Institute Inc, Hayward, Calif), containing total RNA from adults (frontal lobe, temporal lobe, parietal lobe, occipital lobe, cerebellum, and lung). The blots were exposed to autoradiography film (BioMax; Eastman Kodak, Rochester, NY) for 2 hours to overnight.

#### IN SITU HYBRIDIZATION

Fresh frozen tissue sections were processed as described in a previous article.<sup>44</sup> Sections were hybridized with 200  $\mu$ L of hybridization cocktail containing radiolabeled probe, 5 ng/mL, and were exposed to film for 14 days (dysbindin), 2 days (spinophilin and synaptophysin), or 1 day (cyclophilin). For the anatomic survey of dysbindin mRNA levels, areas were sampled by outlining the region of interest. Quantitation of mRNA levels in the DLPFC was performed as previously described elsewhere.<sup>28</sup> We analyzed dysbindin mRNA expression in the pars compacta (most cases were bilaterally sampled) of the mid-brain by outlining the region of robust tyrosine hydroxylase immunohistochemical staining on adjacent tissue slices and by drawing a matching region of interest over the scanned films from the in situ hybridization for dysbindin mRNA.

#### GENOTYPE DETERMINATION

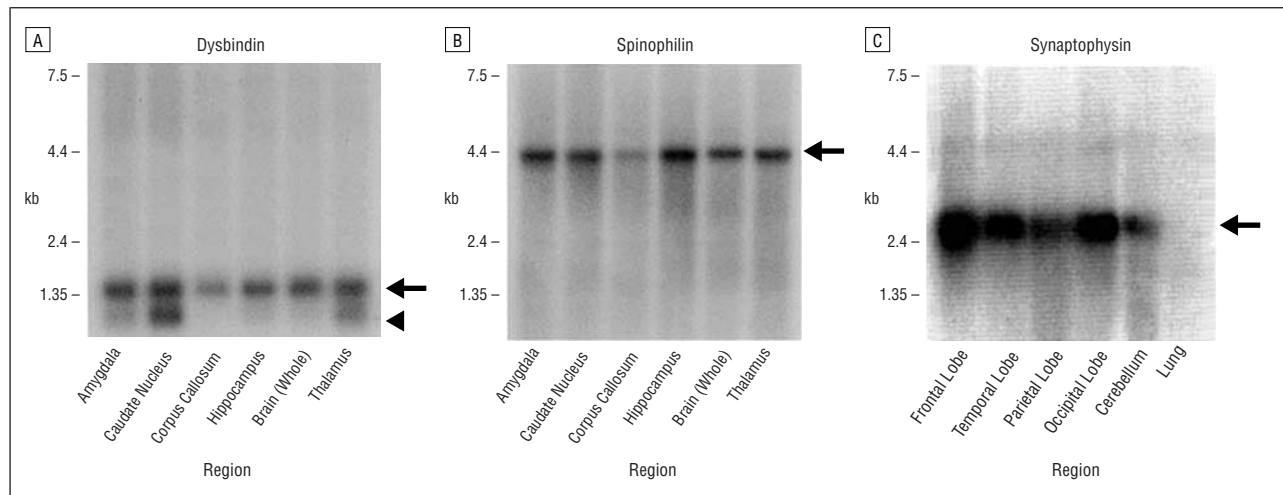
Tissue from the lateral hemisphere of postmortem cerebelli were pulverized and weighed while frozen. DNA was isolated by following the general protocol supplied by PUREGENE (Gentra Systems, Minneapolis, Minn). We determined the genotype at 11 SNPs

using the TaqMan 5' exonuclease allelic discrimination assay.<sup>45</sup> The information for each SNP is given in **Table 2**, in the following column order: our laboratory number, the intermarker distances, the distance from the first SNP, the SNP identification number (either the rs [reference SNP cluster] number from the National Center for Biotechnology Information, Bethesda, Md, or the human Celera Variant number [hCV] from Celera Discovery Systems, Celera Genomics, Rockville, Md), the allelic nucleotides with the common allele shown first, the rare allele frequency, and the SNP location relative to the intron/exon structure of the common 10-exon transcript BC011912, followed by the chromosomal position from the April 2003 freeze (the version of the genomic sequence that this analysis was based on) at the University of California at Santa Cruz (available at: <http://genome.ucsc.edu/>).

#### STATISTICAL ANALYSIS

Statistical comparisons between unaffected controls and patients with schizophrenia were made using an analysis of variance or analysis of covariance followed by post hoc least significant difference or *t* tests, with diagnosis (between-group factor) and cortical layer (within-group factor) as independent variables and mRNA level as the dependent variable. Samples with mRNA levels more than  $\pm 2$  SD from the mean were considered outliers and were omitted from the analysis. Correlations between dysbindin, spinophilin, and synaptophysin mRNA levels and pH, PMI, and age were tested using a Pearson product moment correlation. Either Spearman correlations or Pearson product moment correlations were performed between the estimated neuroleptic exposure level in patients with schizophrenia and mRNA measurements of dysbindin, spinophilin, and synaptophysin. Differences in dysbindin mRNA associated with genotype, regardless of diagnostic category, were analyzed using separate Mann-Whitney *U* tests, with individuals heterozygous and homozygous for the rare allele grouped together. To avoid potential stratification problems due to unequal representation of ethnic groups, only brains from the predominant ethnic group (African American) were analyzed for association between genotype and expression. Therefore, 2 white individuals were dropped from the control group and 3 were dropped from the schizophrenia group (Table 1).





**Figure 1.** Sister Northern blots containing poly A RNA extracted from multiple adult brain regions hybridized with our dysbindin and spinophilin riboprobe. The Northern blot for synaptophysin was run on total RNA extracted from brain regions and the lung. A, For dysbindin, 2 prominent transcripts, one at approximately 1.4 kilobase (kb) (arrow) and another at approximately 1.2 kb (arrowhead), are detected. The longer dysbindin transcript is found in all brain regions studied, and the shorter transcript is seen in the caudate, thalamus, and amygdala. For spinophilin (B) and synaptophysin (C), one major hybridizing band at approximately 4.6 and 2.5 kb (arrows), respectively, are found in all of the brain regions studied. Note that no hybridization of the synaptophysin riboprobe to total RNA from the lung is detected.

## RESULTS

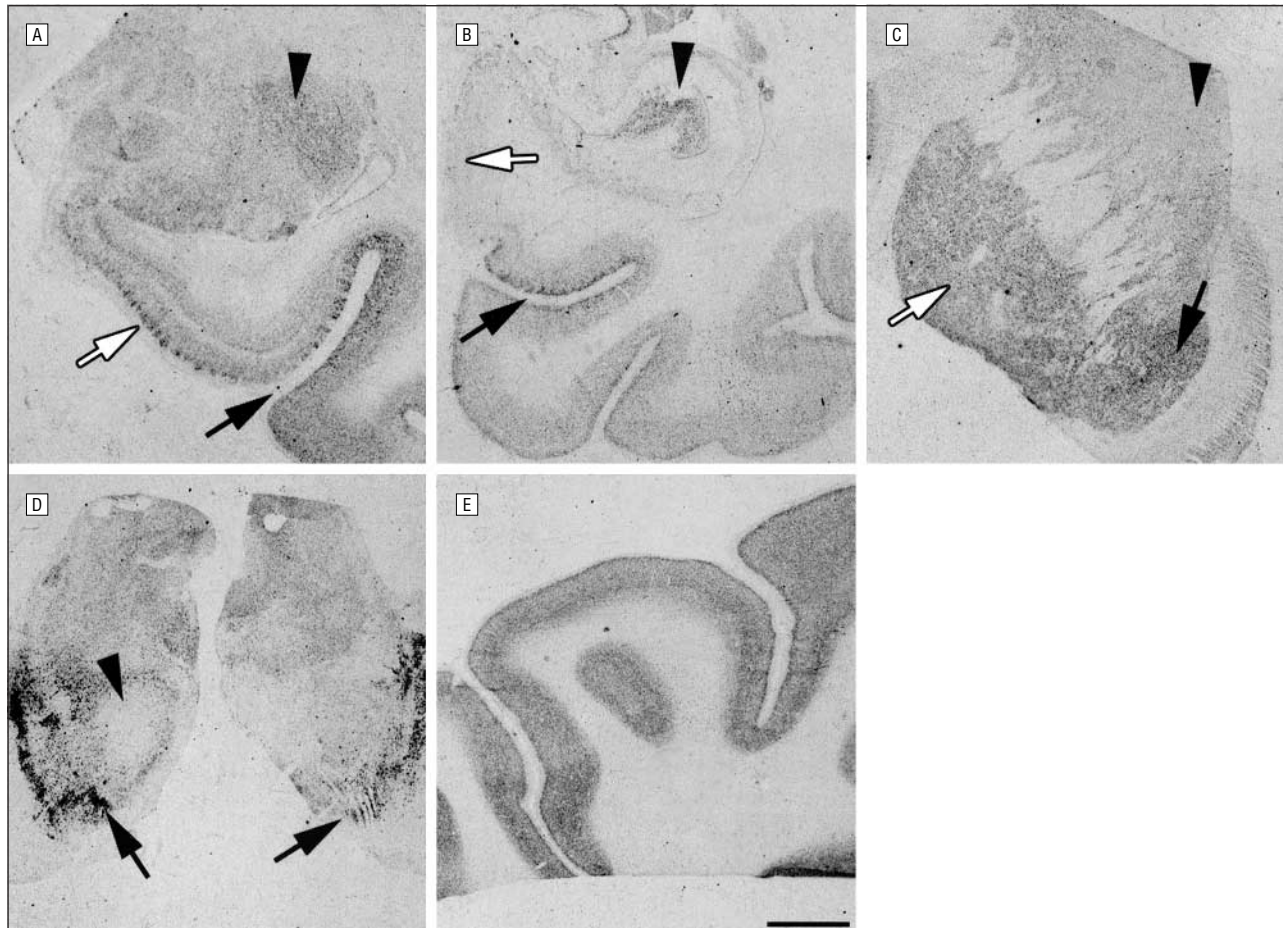
### NORTHERN BLOTTING

By Northern blotting, we detected a band at 1.4-kilobase (kb) transcript size, which probably corresponds to GenBank accession No. BC011912 of dysbindin, in all gray matter areas surveyed: amygdala, caudate nucleus, hippocampus, and thalamus (**Figure 1**). We also detected a band at approximately 1.2 kb in subcortical gray matter areas derived from the telencephalon (caudate and amygdala) and the diencephalon (thalamus), the identity of which is not clear. It is likely that there are several splice variants of dysbindin mRNA of varying abundance that have not been characterized in different brain regions and cell types. Based on the current available data, our probe is predicted to monitor all known common dysbindin mRNA variants in the DLPFC, and it is possible that some rarer transcripts would not be detected. The Northern blot for spinophilin revealed a major band at approximately 4.6 kb in all brain regions examined, consistent with transcript size.<sup>46</sup> Spinophilin mRNA levels were similar across subcortical and cortical areas studied. We detected one major synaptophysin transcript at the expected 2.5-kb size<sup>42,43</sup> in all brain regions examined, with no apparent band in total RNA from the lung.

### ANATOMIC DISTRIBUTION OF DYSBINDIN mRNA IN NORMAL ADULT BRAIN

We detected dysbindin mRNA in multiple regions of the adult brain by using in situ hybridization. No detectable image was observed from sections hybridized with the dysbindin sense strand control riboprobe (data not shown). Overall, dysbindin mRNA was expressed much more prominently in most gray matter areas relative to white matter areas, consistent with dysbindin localization predominantly to neurons and not glia. However, the low white matter dysbin-

din mRNA signal that we detect could reflect either a low dysbindin mRNA expression by glia or a low cell density of dysbindin-positive subcortical interstitial white matter neurons. In cortical areas, dysbindin mRNA signal was most robust in the DLPFC (**Figure 2E**), followed by the temporal neocortex (Figure 2B), the entorhinal cortex (Figure 2A and B), and the orbital frontal cortex. The dysbindin mRNA signal was fairly strong in the entorhinal cortex, where the hybridization varied according to cortical layer, with an intense signal in the superficial cell clusters in layer II, a prominent signal in pyramidal neuronal layer III, no signal in the lamina dissecans, and a strong signal in layers V and VI (Figure 2A). The caudal portion of the entorhinal cortex (Figure 2B) seemed to express less dysbindin mRNA relative to more rostral levels (Figure 2A). The laminar expression of dysbindin mRNA is distinct in the temporal neocortex found lateral to the collateral sulcus and is expressed more abundantly in the superficial cortical layers (layer II) as opposed to deeper cortical layers (Figure 2B). Of the non-cortical regions sampled, dysbindin mRNA was most highly expressed in the substantia nigra of the human midbrain (Figure 2D). A moderate dysbindin hybridization signal was noted in the periaqueductal gray area, the superior colliculus, and the midbrain reticular formation. Dysbindin was clearly expressed in subcortical telencephalic regions and was found at about equal intensities in the amygdala (Figure 2A), hippocampus (Figure 2B), and caudate nucleus (Figure 2C). In the basal ganglia, the nucleus accumbens contained more dysbindin mRNA than the caudate nucleus, whereas the putamen had intermediate levels (Figure 2C). The optical density corresponding to dysbindin mRNA expression varied significantly across anatomic regions ( $F = 5.37$ ;  $P < .01$ ) (**Figure 3**). The gray matter of the medial frontal gyrus (DLPFC) had significantly more dysbindin mRNA than did the frontal white matter ( $P < .001$ ) and the gray matter of the orbital frontal cortex ( $P < .01$ ) (Figure 3A). However, dysbindin mRNA levels were not significantly higher in the DLPFC compared with the other cortical areas sampled



**Figure 2.** Autoradiographic images of dysbindin messenger RNA (mRNA) signal in the brain. A, The rostral mesial temporal lobe with the collateral sulcus (black arrow) of the temporal lobe. The amygdala (arrowhead) and the entorhinal cortex (white arrow) express dysbindin mRNA. B, Coronal section of the mesial temporal lobe at the genu of the hippocampus (arrowhead) with the collateral sulcus (black arrow). Note the light labeling in the caudal entorhinal cortex (white arrow) compared with the distinct labeling found in the more ventrolateral temporal neocortex (black arrow). C, Hybridization of dysbindin riboprobe to the caudate nucleus (arrowhead), putamen (white arrow), and nucleus accumbens (black arrow). D, Intense dysbindin mRNA signal in the substantia nigra (black arrows) of the midbrain with light signal in the red nucleus (arrowhead). E, Strong dysbindin mRNA signal in all cortical layers of the middle frontal gyrus. Scale bar=1 cm.

( $P > .05$  for all). In the mesial temporal lobe, the temporal neocortex has higher dysbindin mRNA levels than the hippocampus ( $P < .01$ ), amygdala ( $P < .05$ ), and caudal entorhinal cortex ( $P = .01$ ) but not the rostral entorhinal cortex (Figure 3B). Dysbindin mRNA was moderately expressed in the nucleus accumbens, where levels were higher than in the caudate ( $P = .01$ ) (Figure 3C), and highly expressed in the substantia nigra, where levels were higher than in the red nucleus and cerebral peduncles ( $P < .05$ ) (Figure 3D).

## DYSBINDIN mRNA LEVELS IN SCHIZOPHRENIA

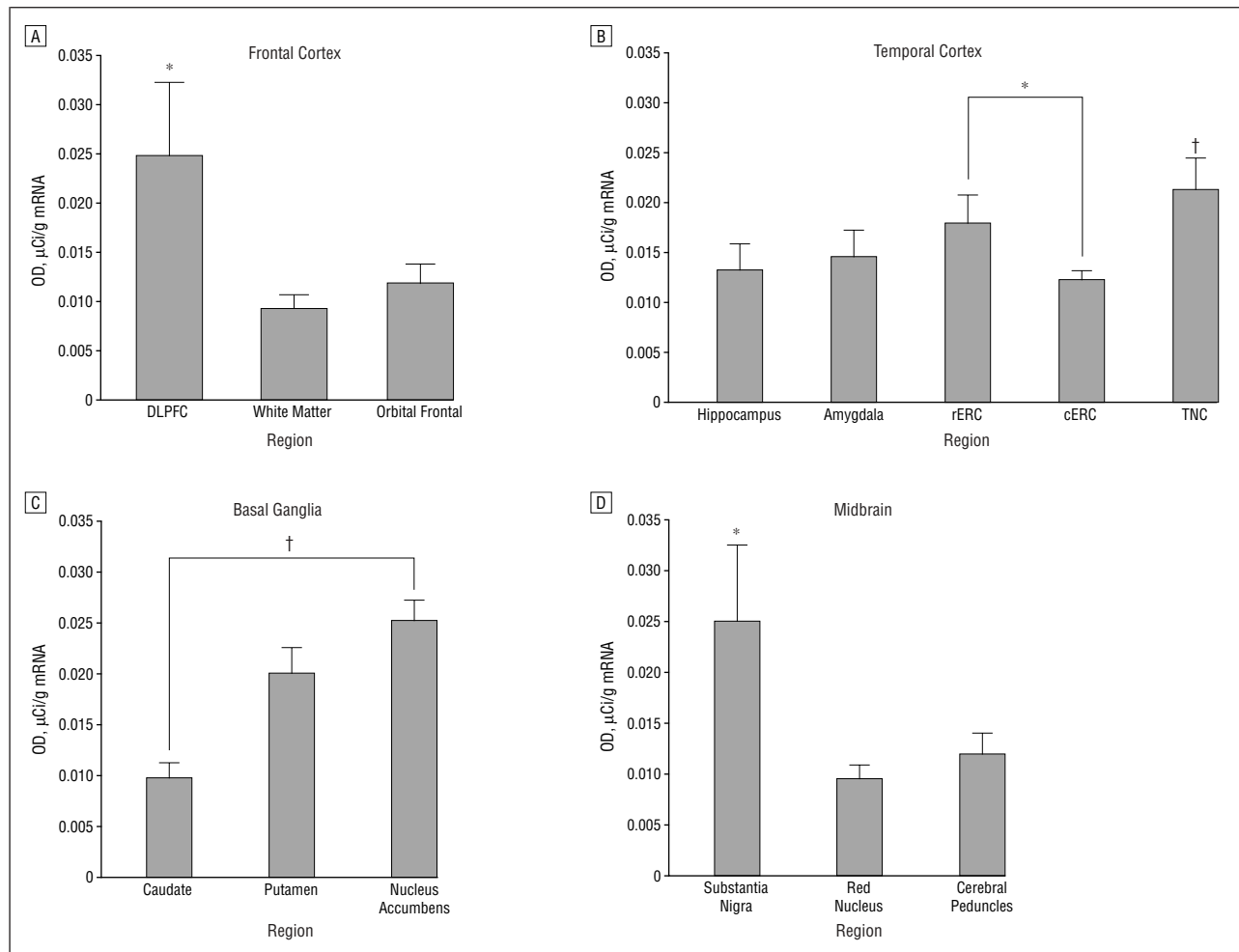
### The DLPFC

Dysbindin mRNA is found in the gray matter of the middle frontal gyrus in patients and controls, with increased signal in the middle to deep cortex (layers IV to VI) (Figure 4A and B). We found clearly distinguishable dysbindin mRNA-positive pyramidal neurons in the DLPFC (data not shown). We detected a significant 15% to 20% reduction in dysbindin mRNA in the brains of patients with schizophrenia compared with controls ( $F_{1,24} = 4.51$ ;  $P = .04$ ) (Figure 5). Furthermore, dysbindin mRNA levels were

found to vary according to cortical depth in both groups (main effect of layer:  $F_{1,120} = 110.84$ ;  $P < .001$ ). Each cortical layer had a significantly different amount of dysbindin compared with the other layers (least significance difference:  $P < .05$  for all) except for layers IV and V, which had similar levels of dysbindin mRNA. We also found a significant interaction effect between diagnostic group and cortical layer ( $F_{5,120} = 6.64$ ;  $P = .03$ ). Post hoc *t* test analysis revealed that dysbindin mRNA levels were reduced in patients in cortical layers with prominent pyramidal neurons, that is, layers II ( $t_{24} = -2.05$ ;  $P = .05$ ), III ( $t_{24} = -2.06$ ;  $P = .05$ ), V ( $t_{24} = -2.26$ ;  $P = .03$ ), and VI ( $t_{24} = -2.14$ ;  $P = .04$ ), whereas they were not significantly changed in layers I ( $t_{24} = 0.37$ ;  $P = .72$ ) and IV ( $t_{24} = -1.59$ ;  $P = .13$ ). Dysbindin mRNA levels did not correlate significantly with age, PMI, or pH in the DLPFC in any cortical layer. Dysbindin mRNA levels did not correlate significantly with any measure of neuroleptic exposure in the DLPFC (average  $r = -0.10$ ;  $P > .16$  for all).

### The Midbrain

We found fairly robust dysbindin mRNA expression in the midbrain of patients with schizophrenia, and the dysbindin



**Figure 3.** Mean optical density (OD) reading from dysbindin messenger RNA (mRNA) signal per anatomic region sampled from autoradiographic images: frontal cortex (A), temporal cortex (B), basal ganglia (C), and midbrain (D). Significant regional differences in dysbindin mRNA levels compared with other measurements on the same graph are found. In the mesial temporal lobe, significant differences are found between the rostral entorhinal cortex (rERC) and the caudal entorhinal cortex (cERC). Asterisk indicates  $P < .05$ ; dagger,  $P = .01$ ; DLPFC, dorsolateral prefrontal cortex; and TNC, temporal neocortex. Error bars represent standard error of the mean.

din expression pattern seemed to be similar to that of controls (Figure 6A and B). On quantifying, patients showed a 26% mean decrease in dysbindin mRNA levels in the substantia nigra (Figure 6C). This mean difference, although similar in magnitude to that found in the DLPFC, showed only a weak trend toward statistical significance using analysis of covariance ( $F_{1,16} = 2.73$ ;  $P = .12$ , with a nondirectional hypothesis). In the midbrain, dysbindin mRNA levels correlated negatively with PMI ( $r = -0.51$ ;  $P < .05$ ) and positively with brain pH ( $r = 0.76$ ;  $P < .001$ ). Hence, these factors were used as covariates in the analysis of covariance for this region. Dysbindin mRNA levels did not significantly correlate with measure of neuroleptic exposure in the midbrain (last dose:  $r = -0.27$ ,  $P = .56$ ; daily dose:  $r = -0.22$ ,  $P = .63$ ; total lifetime dose:  $r = 0.56$ ,  $P = .20$ ).

#### SPINOPHILIN, SYNAPTOPHYSIN, AND CYCLOPHILIN mRNA LEVELS IN SCHIZOPHRENIA

Spinophilin (Figure 4C and D) and synaptophysin (Figure 4E and F) mRNA hybridization signals in the DLPFC were robust and had a laminar appearance, with increased intensity at the middle cortex level (layer effect,

$F > 57$ ;  $P < .001$  for both). In contrast to the reduction in dysbindin mRNA density, we did not detect a significant difference in spinophilin or synaptophysin mRNA levels in the DLPFC of patients with schizophrenia compared with controls ( $F_{1,27} = 0.66$ ;  $P = .42$  and  $F_{1,25} = 0.09$ ;  $P = .77$ , respectively) (Figure 5B and C), and there was no evidence of an alteration in lamina-specific patterns between the groups (interaction effect: spinophilin,  $F_{5,135} = 1.14$ ;  $P = .34$  and synaptophysin,  $F_{5,125} = 0.32$ ;  $P = .90$ ). We found no diagnostic difference in cyclophilin mRNA levels, used as a “housekeeping gene” control for overall mRNA levels (Figure 5D).

Fairly consistent negative correlations between age and spinophilin and synaptophysin mRNA levels were found in controls in layers III, IV, V, and VI ( $r = -0.45$  to  $-0.69$ ;  $P \leq .05$  for all, except for spinophilin mRNA level in layer III, where  $P = .07$ ). No statistically significant correlations between PMI and spinophilin mRNA level or PMI and synaptophysin mRNA level were detected. Spinophilin and synaptophysin mRNA levels correlated with tissue pH in layers III, IV, V, and VI (all-around  $r = -0.40$ ;  $P < .05$  for all). Analyses of covariance with age and pH (spinophilin and synaptophysin) as covariates did not al-



ter the statistical significance of the main effect of diagnosis or the interaction effect. Spinophilin mRNA levels in the DLPFC showed negative correlations with neuroleptic dose estimates (average  $r = -0.44$ ;  $P > .05$  for all). This suggests that neuroleptics could be down-regulating spinophilin mRNA levels, consistent with data<sup>47</sup> showing that long-term treatment with haloperidol significantly decreased spinophilin protein levels in the primate PFC. In general, synaptophysin mRNA levels did not correlate with any measure of neuroleptic exposure (overall average  $r = -0.26$ ;  $P > .05$  for all).

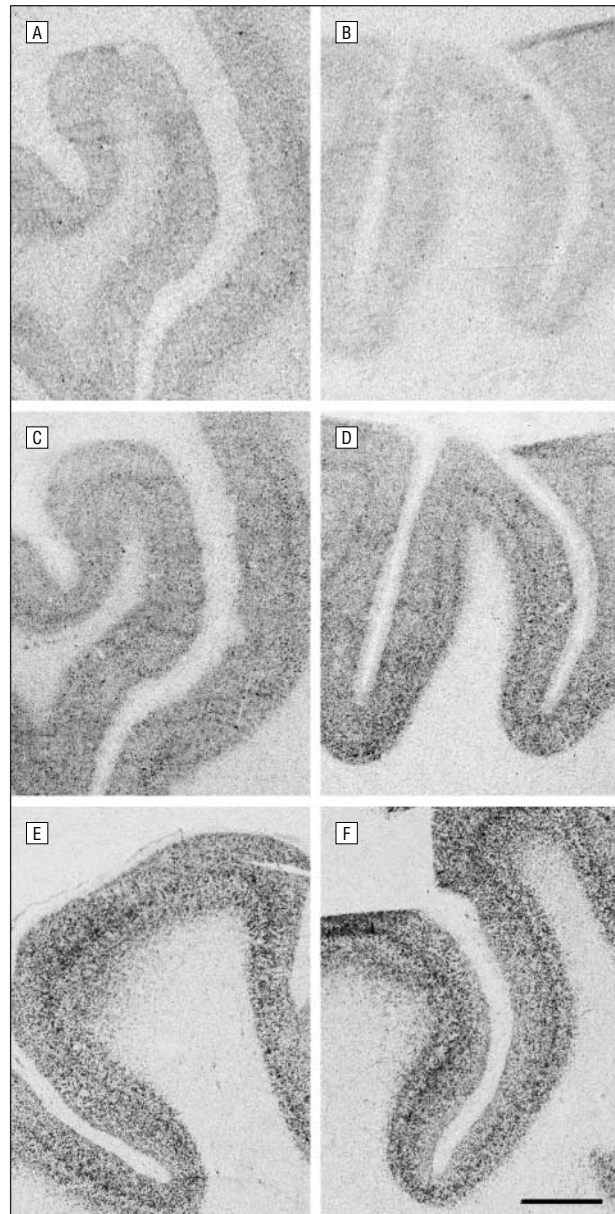
Synaptophysin mRNA levels positively correlated with dysbindin mRNA levels in most cortical layers (II-VI), as did spinophilin mRNA levels; however, the latter correlations reached statistical significance in layer II only (**Table 3**). The positive correlations between dysbindin and synaptophysin were not found when controls were analyzed separately but were found to be strong and statistically significant when patients with schizophrenia were analyzed separately. We also noted that in cortical layers II to VI, statistically significant positive correlations were found between synaptophysin and spinophilin mRNA levels.

#### EFFECT OF DYSBINDIN GENOTYPE ON DYSBINDIN mRNA LEVELS

We grouped individuals heterozygous and homozygous for the rare alleles (ie, 1/2 and 2/2 genotypes, or 2-allele carriers) together to obtain a large enough sample size to allow comparison with individuals homozygous for the common allele (ie, 1/1 genotype). Also, we omitted white individuals from the analysis to avoid comparing groups of mixed ethnicity. Dysbindin mRNA levels in the PFC varied significantly according to genotype at 4 of the 11 SNPs where analysis was possible (Table 2). P3230 is in the 3' UTR, and the G allele carriers showed a 17% increase in expression; P2555 is in intron 3, and the A allele carriers showed a 22% increase in expression; P3521 is in the 5' UTR, and the G allele carriers showed a 34% decrease in expression; and P3587 is in the 5' flanking region of the gene, and the C allele carriers showed a 39% increase in expression.

#### COMMENT

We detected abundant and widespread expression of dysbindin mRNA in the adult brain. The presence of dysbindin in cortical and subcortical regions of the normal brain has recently been reported at the protein level as well.<sup>48</sup> We found that the dysbindin mRNA level is reduced in the DLPFC and possibly in the substantia nigra of patients with schizophrenia. No change in spinophilin, synaptophysin, or cyclophilin mRNA levels in the DLPFC of patients with schizophrenia was detected. These findings suggest that the reduction in dysbindin mRNA levels is not secondary to an overall reduction in mRNA abundance or quality. Neither is it likely to be simply the result of a generalized loss of synaptic contacts. Our preliminary results of a reduction in dysbindin mRNA levels in the substantia nigra, although this did not reach statistical significance, suggest that reduc-

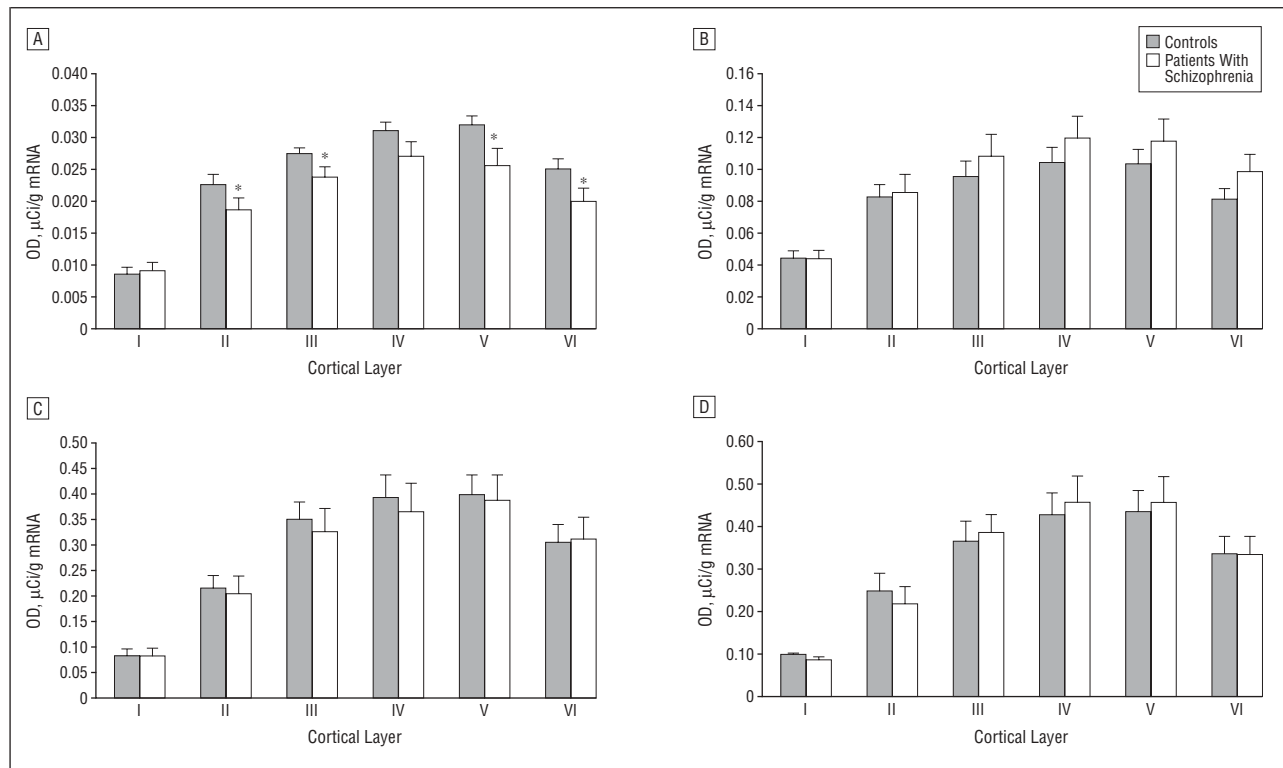


**Figure 4.** Autoradiographic images of dysbindin (A and B), spinophilin (C and D), and synaptophysin (E and F) messenger RNA (mRNA) in the dorsolateral prefrontal cortex. Adjacent (not always serial) sections from 2 control cases (A and C are from one case and E is from another case) and from a single schizophrenic case (B, D, and F) are shown. Patients with schizophrenia have a similar but overall less intense pattern of dysbindin mRNA expression compared with controls. Spinophilin mRNA and synaptophysin mRNA are most abundant in the middle cortical layers of healthy individuals and of schizophrenic patients with no apparent diminution of signal. Scale bar = 1 cm.

tion in dysbindin synthesis in patients may not be restricted to the DLPFC, a fact that is also supported by another recent finding of reduced dysbindin protein levels in the hippocampus of patients with schizophrenia relative to controls.<sup>49</sup>

Dysbindin mRNA was expressed in temporal and frontal cortical association areas involved in declarative memory (the hippocampus and entorhinal cortex) and working memory (the DLPFC). Abnormalities in hippocampal-based memory are commonly found in patients with schizophrenia, and abnormalities in DLPFC





**Figure 5.** Mean optical density (OD) of dysbindin (A), spinophilin (B), synaptophysin (C), and cyclophilin (D) messenger RNA (mRNA) plotted according to cortical layer. Patients with schizophrenia express less dysbindin mRNA in cortical layers II, III, V, and VI vs controls. Asterisk indicates  $P < .05$ . Error bars represent standard error of the mean.

function are considered one of the core cognitive problems in patients with schizophrenia.<sup>26</sup> Furthermore, ample evidence<sup>25,50-52</sup> of cellular and molecular pathology exists in the temporal and frontal cortices in brains of patients with schizophrenia. Dysbindin mRNA is prominently expressed in the substantia nigra and basal ganglia, areas of origination and termination of dopamine neurons, suggesting that dysbindin dysfunction may affect regions with prominent dopamine neurotransmission. Dysbindin gene expression was also detected in the amygdala, a brain area that has received less attention in pathology research in schizophrenia but that is implicated because of aberrant emotional responses in patients.<sup>53-55</sup> Overall, we conclude that some parallels between the anatomic expression pattern of dysbindin and the vulnerability of these regions to functional pathology in schizophrenia can be drawn.

Reduced dysbindin expression may directly relate to the synaptic pathology in the schizophrenic DLPFC; however, it is not clear if it lies upstream or downstream of the putatively altered synaptic communication. Certainly, some models of cortical pathology in schizophrenia include altered synaptic communication among cortical neurons as a component of disease etiology.<sup>35</sup> In contrast, we and other researchers<sup>56-58</sup> have failed to find a reduction in synaptophysin or spinophilin mRNA or protein levels in the DLPFC of patients with schizophrenia, emphasizing that a generalized decrease in the number or density of synaptic contacts in the frontal cortex may not be readily detectable in all cohorts. However, we found a statistically significant positive correlation be-

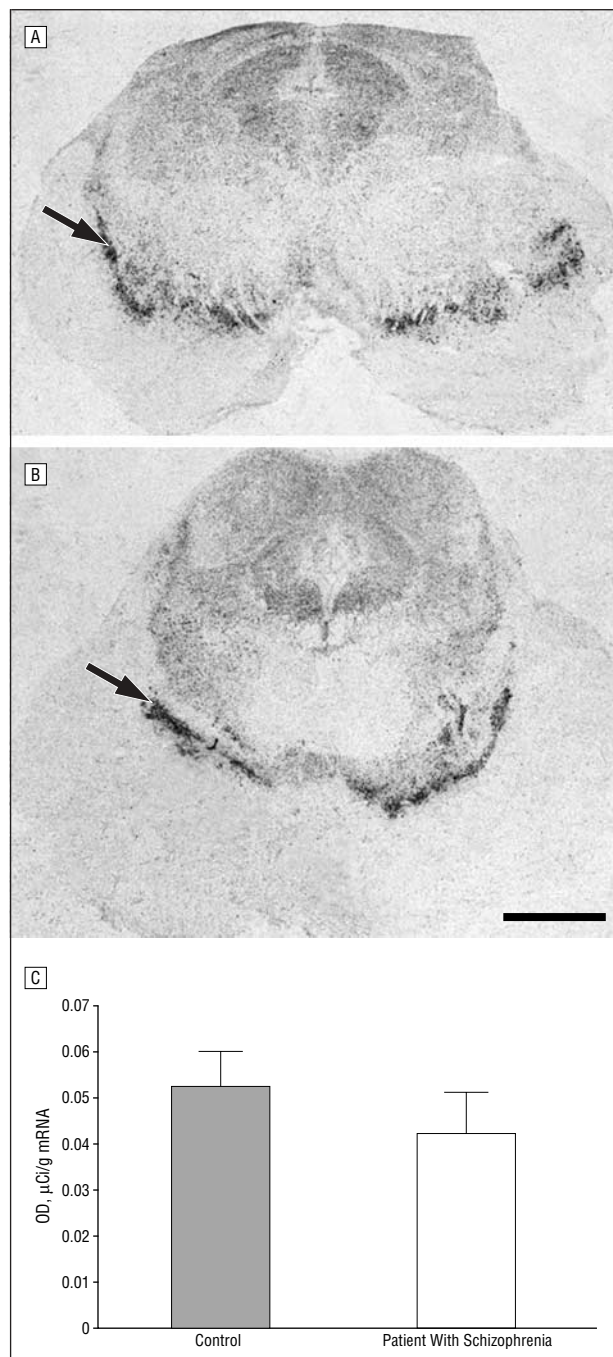
tween dysbindin and synaptophysin mRNA levels in the DLPFC of patients with schizophrenia, suggesting that there could be a link between dysbindin and synaptophysin mRNA levels in the pathological state. Our results suggest that synaptic pathology may be difficult to detect by gross measurements in overall group comparisons with small numbers of subjects, and, in agreement with other researchers in the field, we suggest that synaptic pathology may be subtle and variable and may be due to subject-specific etiologic factors in the DLPFC of patients with schizophrenia.<sup>35</sup>

Although specific SNPs in dysbindin have been associated with schizophrenia through genetic analysis, it is unlikely that any of the causative "mutations" have been identified. In 2 of the published studies with positive results,<sup>12,13</sup> different alleles are overtransmitted to affected individuals at some SNPs. These differences indicate that the SNPs tested are in linkage disequilibrium with the unknown causative variants. Our observation that the dysbindin mRNA level is reduced in patients with schizophrenia may be due, in part, to such causative variants in the dysbindin gene. Dysbindin mRNA may also be down-regulated in the patients studied herein by the action of other schizophrenia-susceptibility genes or by environmental variables related to schizophrenia. In this study, sequence variations in the 3' UTR, in intron 3, in the 5' UTR, and in the 5' flanking region (a putative promoter region) of the dysbindin gene all significantly impacted dysbindin mRNA levels in the PFC. Most of our results concerning the effects of genotype on expression should be viewed as preliminary, as we had a small num-

ber of subjects in many of the comparisons. However, our finding with SNP P3230 (called 1580740, a→g, in Bray et al<sup>59</sup>), where inheritance of the G allele predicts increased dysbindin mRNA levels, is consistent with a previous study of the same SNP by Bray and colleagues<sup>59</sup> using allele-specific expression analysis. In their study, using heterozygous individuals, dysbindin mRNA transcripts containing the G allele at this SNP are increased compared with dysbindin mRNA transcripts with the A allele, but to a variable degree depending on the individual. For the other SNP (called 15643772, t→c, in Bray et al<sup>59</sup>), which corresponds to our P3236, a smaller difference in allele-specific expression was found, but we did not find any effect of this SNP on dysbindin mRNA levels in our overall group analysis.

The function of dysbindin in the mammalian brain is not well understood, so it is difficult to predict how, if at all, a modest reduction in dysbindin mRNA would be detrimental to the brain. Dysbindin binds to dystrobrevins, key components of the DPC, a protein complex that links the extracellular matrix to the intracellular cytoskeleton.<sup>19</sup> The DPC can contain dystrophins, syntrophins, dystroglycans, and  $\alpha$ - and  $\beta$ -dystrobrevins, as well as other proteins that are localized to pyramidal neurons in the rodent cerebral cortex and hippocampus.<sup>21,60,61</sup> Many proteins of the DPC are enriched in postsynaptic densities of inhibitory synapses and are not detected in postsynaptic sites of excitatory glutamate synapses.<sup>62</sup> In cell culture, dystrophin has been shown to extensively co-localize with  $\alpha$ -2- $\gamma$ -aminobutyric acid (GABA)(A) receptors opposite presynaptic terminals containing GABA.<sup>62</sup> Other DPC components, syntrophin, and  $\beta$ -dystroglycan also cluster at GABAergic synapses, suggesting that the entire DPC may assemble at postsynaptic inhibitory sites. It is possible that the DPC complex plays a unique role at these inhibitory synaptic sites and that this role may be altered in schizophrenia. Indeed, ample evidence for altered GABAergic neurotransmission in schizophrenia can be found.<sup>51</sup> However, further work localizing dysbindin mRNA and protein at the cellular and subcellular levels is needed to determine whether dysbindin is localized to certain subsets of neurons or terminals in the primate cortex. Also, additional studies aimed at determining the function(s) of dysbindin in brain cells are needed. Indeed, a recent study<sup>23</sup> shows that dysbindin binds to proteins of a multimeric complex distinct from the DPC—the BLOC-1 (biogenesis of lysosome-related organelles complex 1)—and that dysbindin is mutated in a human disease associated with impairment of organelle trafficking, stressing that our knowledge of the cellular function of dysbindin in the brain is rudimentary.

Alterations in dysbindin should ultimately relate to the documented abnormalities in excitatory neurotransmission of the DLPFC in schizophrenia, although direct evidence of this is currently lacking. Glutamate neuronal plasticity and activity in the schizophrenic PFC are altered, and molecular indications of these alterations include a reduction in *N*-acetyl aspartate levels,<sup>63-66</sup> a reduction in BDNF mRNA and protein levels,<sup>29</sup> and a reduction in GAP-43 mRNA levels.<sup>28</sup> In addition, in the same set of brains studied herein, we found sta-



**Figure 6.** Robust dysbindin messenger RNA (mRNA) is detected in the substantia nigra of controls (A) and patients with schizophrenia (B) (arrows). Scale bar=1 cm. C, Mean optical density (OD) corresponding to dysbindin mRNA levels in the substantia nigra. Error bars represent standard error of the mean.

tistically significant reductions in mRNA encoding neurotrophin receptors, which localize to spines, which are specialized sites for excitatory signaling.<sup>67</sup> In fact, reductions in mRNA encoding spine-related postsynaptic proteins, such as PSD95 and synapse-associated protein-97,<sup>68,69</sup> and reductions in spine density<sup>70,71</sup> have been found in the PFC of patients with schizophrenia by other research groups. These studies, taken together, with the possible lack of change in spinophilin levels, suggest that many, but not all, aspects of the postsynap-

**Table 3. Correlations of Dysbindin, Spinophilin, and Synaptophysin Messenger RNA Levels in the Dorsolateral Prefrontal Cortex in All Subjects by Cortical Layer**

Cortical Layer	Pearson Product Moment Correlation, <i>r</i> ( <i>P</i> Value)	
	Spinophilin	Synaptophysin
II		
Dysbindin	0.41 (.04)	0.20 (.35)
Synaptophysin	0.37 (.07)	...
III		
Dysbindin	0.37 (.07)	0.51 (.01)
Synaptophysin	0.42 (.04)	...
IV		
Dysbindin	0.39 (.06)	0.64 (<.001)
Synaptophysin	0.42 (.04)	...
V		
Dysbindin	0.36 (.08)	0.68 (<.001)
Synaptophysin	0.46 (.02)	...
VI		
Dysbindin	0.34 (.10)	0.47 (.02)
Synaptophysin	0.49 (.01)	...

tic spine may be dysfunctional in the brains of patients with schizophrenia. The possibility that abnormalities in glutamate-mediated excitatory neurotransmission may be due to dysbindin-related pathology is the subject of ongoing research.

In summary, we report robust dysbindin mRNA expression in multiple brain areas implicated in the pathology of schizophrenia, including the frontal and temporal cortical regions and subcortical sites. We found reduced expression of dysbindin in the DLPFC and possibly in the midbrain of patients with schizophrenia. We report preliminary observations on the relationship between dysbindin mRNA levels and genetic variation in the dysbindin gene. Further molecular genetic analysis of dysbindin gene variants may prove informative because the control of dysbindin gene transcription or of dysbindin transcript stability seems to be altered in the brains of patients with schizophrenia. Further studies are required to determine whether a reduction in the dysbindin mRNA level is specific to schizophrenia or extends to patients with other severe mental illnesses. This study provides initial insights into a molecular mechanism of disease pathology in schizophrenia using the evidence of dysbindin as a schizophrenia-susceptibility gene as a starting point to determine where in the brain the dysbindin gene is transcribed and to identify dysbindin reduction at mRNA levels as part of the molecular abnormalities associated with schizophrenia.

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Corresponding author and reprints: Cynthia Shannon Weickert, PhD, Intramural Research Program, National In-

stitute of Mental Health, National Institutes of Health, 10 Center Dr, Bldg 10, Room 4N312, Mail Stop Code 1385, Bethesda, MD 20892-1385 (e-mail: shannowc@intr.nimh.nih.gov).

## REFERENCES

- Cloninger CR. The discovery of susceptibility genes for mental disorders. *Proc Natl Acad Sci U S A*. 2002;99:13365-13367.
- Harrison PJ, Owen MJ. Genes for schizophrenia? recent findings and their pathophysiological implications. *Lancet*. 2003;361:417-419.
- Mowry BJ, Nancarrow DJ. Molecular genetics of schizophrenia. *Clin Exp Pharmacol Physiol*. 2001;28:66-69.
- Weinberger DR, Egan MF, Bertolino A, Callicott JH, Mattay VS, Lipska BK, Berman KF, Goldberg TE. Prefrontal neurons and the genetics of schizophrenia. *Biol Psychiatry*. 2001;50:825-844.
- Bunney WE, Bunney BG. Evidence for a compromised dorsolateral prefrontal cortical parallel circuit in schizophrenia. *Brain Res Brain Res Rev*. 2000;31:138-146.
- Jones LB. Recent cytoarchitectonic changes in the prefrontal cortex of schizophrenics. *Front Biosci*. 2001;6:E148-E153.
- Lewis DA, Gonzalez-Burgos G. Intrinsic excitatory connections in the prefrontal cortex and the pathophysiology of schizophrenia. *Brain Res Bull*. 2000;52:309-317.
- Selemon LD, Goldman-Rakic PS. The reduced neuropil hypothesis: a circuit based model of schizophrenia. *Biol Psychiatry*. 1999;45:17-25.
- Costa E, Davis J, Grayson DR, Guidotti A, Pappas GD, Pesold C. Dendritic spine hypoplasticity and downregulation of reelin and GABAergic tone in schizophrenia vulnerability. *Neurobiol Dis*. 2001;8:723-742.
- Sillitoe RV, Benson MA, Blake DJ, Hawkes R. Abnormal dysbindin expression in cerebellar mossy fiber synapses in the mdx mouse model of Duchenne muscular dystrophy. *J Neurosci*. 2003;23:6576-6585.
- Benson MA, Newey SE, Martin-Rendon E, Hawkes R, Blake DJ. Dysbindin, a novel coiled-coil-containing protein that interacts with the dystrobrevins in muscle and brain. *J Biol Chem*. 2001;276:24232-24241.
- Straub RE, Jiang Y, MacLean CJ, Ma Y, Webb BT, Myakishev MV, Harris-Kerr C, Wormley B, Sadek H, Kadambi B, Cesare AJ, Gibberman A, Wang X, O'Neill FA, Walsh D, Kendler KS. Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. *Am J Hum Genet*. 2002;71:337-348.
- Schwab SG, Knapp M, Mondabon S, Hallmayer J, Borrmann-Hassenbach M, Albus M, Lerer B, Rietschel M, Trixler M, Maier W, Wildenauer DB. Support for association of schizophrenia with genetic variation in the 6p22.3 gene, dysbindin, in sib-pair families with linkage and in an additional sample of triad families. *Am J Hum Genet*. 2003;72:185-190.
- Tang JX, Zhou J, Fan JB, Li XW, Shi YY, Gu NF, Feng GY, Xing YL, Shi JG, He L. Family-based association study of DTNBP1 in 6p22.3 and schizophrenia. *Mol Psychiatry*. 2003;8:717-718.
- Hudson TJ. Wanted: regulatory SNPs. *Nat Genet*. 2003;33:439-440.
- Watkins SC, Cullen MJ, Hoffman EP, Billington L. Plasma membrane cytoskeleton of muscle: a fine structural analysis. *Microsc Res Tech*. 2000;48:131-141.
- Grady RM, Zhou H, Cunningham JM, Henry MD, Campbell KP, Sanes JR. Maturation and maintenance of the neuromuscular synapse: genetic evidence for roles of the dystrophin-glycoprotein complex. *Neuron*. 2000;25:279-293.
- Adams ME, Kramarcy N, Krall SP, Rossi SG, Rotundo RL, Sealock R, Froehner SC. Absence of  $\alpha$ -synthrophin leads to structurally aberrant neuromuscular synapses deficient in utrophin. *J Cell Biol*. 2000;150:1385-1398.
- Blake DJ, Kroger S. The neurobiology of Duchenne muscular dystrophy: learning lessons from muscle? *Trends Neurosci*. 2000;23:92-99.
- Claudepierre T, Dalloz C, Mornet D, Matsumura K, Sahel J, Rendon A. Characterization of the intermolecular associations of the dystrophin-associated glycoprotein complex in retinal Muller glial cells. *J Cell Sci*. 2000;113(pt 19):3409-3417.
- Moukhles H, Carbonetto S. Dystroglycan contributes to the formation of multiple dystrophin-like complexes in brain. *J Neurochem*. 2001;78:824-834.
- Peters MF, O'Brien KF, Sadoulet-Puccio HM, Kunkel LM, Adams ME, Froehner SC.  $\beta$ -dystrobrevin, a new member of the dystrophin family: identification, cloning, and protein associations. *J Biol Chem*. 1997;272:31561-31569.
- Li W, Zhang Q, Oiso N, Novak E, Gautam R, O'Brien E, Tinsley C, Blake D, Spritz R, Copelan N, Jenkins N, Amato D, Roe B, Starcevic M, Dell'Angiella E, Elliott R, Mishra V, Kingsmore S, Paylor R, Swank R. Hermansky-Pudlak syndrome type 7 (HPS-7) results from mutant dysbindin, a member of the biogenesis of lysosome-related organelles complex 1 (BLOC-1). *Nat Genet*. 2003;35:84-89.
- Blank M, Blake DJ, Kroger S. Molecular diversity of the dystrophin-like protein complex in the developing and adult avian retina. *Neuroscience*. 2002;111:259-273.
- Weickert CS, Kleinman JE. The neuroanatomy and neurochemistry of schizophrenia. *Psychiatr Clin North Am*. 1998;21:57-75.
- Weickert TW, Goldberg TE, Gold JM, Bigelow LB, Egan MF, Weinberger DR. Cognitive impairments in patients with schizophrenia displaying preserved and compromised intellect. *Arch Gen Psychiatry*. 2000;57:907-913.
- Weinberger DR, Berman KF. Prefrontal function in schizophrenia: confounds and controversies. *Philos Trans R Soc Lond B Biol Sci*. 1996;351:1495-1503.
- Weickert CS, Webster MJ, Hyde TM, Herman MM, Bachus SE, Bali G, Wein-



- berger DR, Kleinman JE. Reduced GAP-43 mRNA in dorsolateral prefrontal cortex of patients with schizophrenia. *Cereb Cortex*. 2001;11:136-147.
29. Weickert CS, Hyde TM, Lipska BK, Herman MM, Weinberger DR, Kleinman JE. Reduced brain derived neurotrophic factor in prefrontal cortex of patients with schizophrenia. *Mol Psychiatry*. 2003;8:592-610.
  30. Thompson PM, Sower AC, Perrone-Bizzozero NI. Altered levels of the synaptosomal associated protein SNAP-25 in schizophrenia. *Biol Psychiatry*. 1998;43:239-243.
  31. Selemon LD, Rajkowska G, Goldman-Rakic PS. Abnormally high neuronal density in the schizophrenic cortex: a morphometric analysis of prefrontal area 9 and occipital area 17. *Arch Gen Psychiatry*. 1995;52:805-818.
  32. Selemon LD, Rajkowska G, Goldman-Rakic PS. Elevated neuronal density in prefrontal area 46 in brains from schizophrenic patients: application of a three-dimensional, stereologic counting method. *J Comp Neurol*. 1998;392:402-412.
  33. Rajkowska G, Selemon LD, Goldman-Rakic PS. Neuronal and glial somal size in the prefrontal cortex: a postmortem morphometric study of schizophrenia and Huntington disease. *Arch Gen Psychiatry*. 1998;55:215-224.
  34. Pierri JN, Volk CL, Auh S, Sampson A, Lewis DA. Decreased somal size of deep layer 3 pyramidal neurons in the prefrontal cortex of subjects with schizophrenia. *Arch Gen Psychiatry*. 2001;58:466-473.
  35. Mirnics K, Middleton FA, Marquez A, Lewis DA, Levitt P. Molecular characterization of schizophrenia viewed by microarray analysis of gene expression in prefrontal cortex. *Neuron*. 2000;28:53-67.
  36. Karson CN, Mrak RE, Schluterman KO, Sturmer WQ, Sheng JG, Griffin WS. Alterations in synaptic proteins and their encoding mRNAs in prefrontal cortex in schizophrenia: a possible neurochemical basis for "hypofrontality." *Mol Psychiatry*. 1999;4:39-45.
  37. Honer WG, Falkai P, Chen C, Arango V, Mann JJ, Dwork AJ. Synaptic and plasticity-associated proteins in anterior frontal cortex in severe mental illness. *Neuroscience*. 1999;91:1247-1255.
  38. Kleinman JE, Hyde TM, Herman MM. Methodological issues in the neuropathology of mental illness. In: Bloom FE, Kupfer DJ, eds. *Psychopharmacology: The Fourth Generation of Progress*. New York, NY: Raven Press Ltd; 1995:859-864.
  39. Zalcman S, Endicott J, Clayton P, Winokur G. *Diagnostic Evaluation After Death (DEAD)*. Rockville, Md: National Institute of Mental Health; 1983.
  40. Keks NA, Hill C, Opeskin K, Copolov D, Dean B. Psychiatric diagnosis after death: the problems of accurate diagnosis from case history review and relative interviews. In: Dean B, Kleinman JE, eds. *Using CNS Tissue in Psychiatric Research: A Practical Guide*. Amsterdam, the Netherlands: Overseas Publishers Association; 1999:19-37.
  41. Matsumoto M, Hidaka K, Tada S, Tasaki Y, Yamaguchi T. Full-length cDNA cloning and distribution of human dopamine D4 receptor. *Brain Res Mol Brain Res*. 1995;29:157-162.
  42. Ozcelik T, Lafreniere RG, Archer BT III, Johnston PA, Willard HF, Francke U, Sudhof TC. Synaptophysin: structure of the human gene and assignment to the X chromosome in man and mouse. *Am J Hum Genet*. 1990;47:551-561.
  43. Sudhof TC, Lottspeich F, Greengard P, Mehl E, Jahn R. The cDNA and derived amino acid sequences for rat and human synaptophysin. *Nucleic Acids Res*. 1987;15:9607.
  44. Whitfield HJ Jr, Brady LS, Smith MA, Mamalaki E, Fox RJ, Herkenham M. Optimization of cRNA probe in situ hybridization methodology for localization of glucocorticoid receptor mRNA in rat brain: a detailed protocol. *Cell Mol Neurobiol*. 1990;10:145-157.
  45. McGuigan FE, Ralston SH. Single nucleotide polymorphism detection: allelic discrimination using TaqMan. *Psychiatr Genet*. 2002;12:133-136.
  46. Allen PB, Ouimet CC, Greengard P. Spinophilin, a novel protein phosphatase 1 binding protein localized to dendritic spines. *Proc Natl Acad Sci U S A*. 1997;94:9956-9961.
  47. Lidow MS, Song ZM, Castner SA, Allen PB, Greengard P, Goldman-Rakic PS. Antipsychotic treatment induces alterations in dendrite- and spine-associated proteins in dopamine-rich areas of the primate cerebral cortex. *Biol Psychiatry*. 2001;49:1-12.
  48. Arnold S, Talbot K, Eidem W, Benson M, Trojanowski J, Blake D, Uryu K. Dysbindin distribution in the brain of rodents and humans. In: *2003 Abstract Viewer/Itinerary Planner*. Washington, DC: Society for Neuroscience; 2003. Program No. 447.4.
  49. Talbot K, Eidem W, Benson M, Smith R, Trojanowski J, Gur R, Hahn C, Blake D, Arnold S. Dysbindin and vesicular glutamate transporter-1 (VGLUT-1) are altered in the hippocampal formation in schizophrenia. In: *2003 Abstract Viewer/Itinerary Planner*. Washington, DC: Society for Neuroscience; 2003. Program No. 447.5.
  50. Harrison PJ, Eastwood SL. Neuropathological studies of synaptic connectivity in the hippocampal formation in schizophrenia. *Hippocampus*. 2001;11:508-519.
  51. Blum PB, Mann JJ. The GABAergic system in schizophrenia. *Int J Neuropsychopharmacol*. 2002;5:159-179.
  52. Weinberger DR, Berman KF, Suddath R, Torrey EF. Evidence of dysfunction of a prefrontal-limbic network in schizophrenia: a magnetic resonance imaging and regional cerebral blood flow study of discordant monozygotic twins. *Am J Psychiatry*. 1992;149:890-897.
  53. Grossberg S. The imbalanced brain: from normal behavior to schizophrenia. *Biol Psychiatry*. 2000;48:81-98.
  54. Kosaka H, Omori M, Murata T, Iidaka T, Yamada H, Okada T, Takahashi T, Sadatao N, Itoh H, Yonekura Y, Wada Y. Differential amygdala response during facial recognition in patients with schizophrenia: an fMRI study. *Schizophr Res*. 2002;57:87-95.
  55. Gur RE, McGrath C, Chan RM, Schroeder L, Turner T, Turetsky BI, Kohler C, Al-sop D, Maldjian J, Ragland JD, Gur RC. An fMRI study of facial emotion processing in patients with schizophrenia. *Am J Psychiatry*. 2002;159:1992-1999.
  56. Koh PO, Bergson C, Undie AS, Goldman-Rakic PS, Lidow MS. Up-regulation of the D1 dopamine receptor-interacting protein, calcyon, in patients with schizophrenia. *Arch Gen Psychiatry*. 2003;60:311-319.
  57. Halim ND, Weickert CS, McClintock BW, Hyde TM, Weinberger DR, Kleinman JE, Lipska BK. Presynaptic proteins in the prefrontal cortex of patients with schizophrenia and rats with abnormal prefrontal development. *Mol Psychiatry*. 2003;8:797-810.
  58. Glantz LA, Austin MC, Lewis DA. Normal cellular levels of synaptophysin mRNA expression in the prefrontal cortex of subjects with schizophrenia. *Biol Psychiatry*. 2000;48:389-397.
  59. Bray NJ, Buckland PR, Owen MJ, O'Donovan MC. Cis-acting variation in the expression of a high proportion of gene in human brain. *Hum Genet*. 2003;113:149-153.
  60. Gorecki DC, Monaco AP, Derry JM, Walker AP, Barnard EA, Barnard PJ. Expression of four alternative dystrophin transcripts in brain regions regulated by different promoters. *Hum Mol Genet*. 1992;1:505-510.
  61. Gorecki DC, Derry JM, Barnard EA. Dystroglycan: brain localisation and chromosome mapping in the mouse. *Hum Mol Genet*. 1994;3:1589-1597.
  62. Brunig I, Suter A, Knesel I, Luscher B, Fritschy JM. GABAergic terminals are required for postsynaptic clustering of dystrophin but not of GABA(A) receptors and gephyrin. *J Neurosci*. 2002;22:4805-4813.
  63. Tsai G, Passani LA, Slusher BS, Carter R, Baer L, Kleinman JE, Coyle JT. Abnormal excitatory neurotransmitter metabolism in schizophrenic brains. *Arch Gen Psychiatry*. 1995;52:829-836.
  64. Block W, Bayer TA, Tepest R, Traber F, Rietschel M, Muller DJ, Schulze TG, Honer WG, Maier W, Schild HH, Falkai P. Decreased frontal lobe ratio of N-acetyl aspartate to choline in familial schizophrenia: a proton magnetic resonance spectroscopy study. *Neurosci Lett*. 2000;289:147-151.
  65. Bertolino A, Callicott JH, Elman I, Mattay VS, Tedeschi G, Frank JA, Breier A, Weinberger DR. Regionally specific neuronal pathology in untreated patients with schizophrenia: a proton magnetic resonance spectroscopic imaging study. *Biol Psychiatry*. 1998;43:641-648.
  66. Bertolino A, Nawroz S, Mattay VS, Barnett AS, Duyn JH, Moonen CT, Frank JA, Tedeschi G, Weinberger DR. Regionally specific pattern of neurochemical pathology in schizophrenia as assessed by multislice proton magnetic resonance spectroscopic imaging. *Am J Psychiatry*. 1996;153:1554-1563.
  67. Weickert CS, Ligons DL, Romanczyk T, Ungaro G, Hyde TM, Herman M, Weinberger DR, Kleinman JE. Neurotrophin receptor mRNA in patients with schizophrenia: insights into cellular pathology of the prefrontal cortex. *Mol Psychiatry*. In press.
  68. Ohnuma T, Kato H, Arai H, Faull RL, McKenna PJ, Emson PC. Gene expression of PSD95 in prefrontal cortex and hippocampus in schizophrenia. *Neuroreport*. 2000;11:3133-3137.
  69. Toyooka K, Iritani S, Makifuchi T, Shirakawa O, Kitamura N, Maeda K, Nakamura R, Niizato K, Watanabe M, Kakita A, Takahashi H, Someya T, Nawa H. Selective reduction of a PDZ protein, SAP-97, in the prefrontal cortex of patients with chronic schizophrenia. *J Neurochem*. 2002;83:797-806.
  70. Glantz LA, Lewis DA. Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Arch Gen Psychiatry*. 2000;57:65-73.
  71. Garey LJ, Ong WY, Patel TS, Kanani M, Davis A, Mortimer AM, Barnes TR, Hirsch SR. Reduced dendritic spine density on cerebral cortical pyramidal neurons in schizophrenia. *J Neurol Neurosurg Psychiatry*. 1998;65:446-453.