Gene Expression Profile for Schizophrenia

Discrete Neuron Transcription Patterns in the Entorhinal Cortex

Scott E. Hemby, PhD; Stephen D. Ginsberg, PhD; Brian Brunk, PhD; Steven E. Arnold, MD; John Q. Trojanowski, MD, PhD; James H. Eberwine, PhD

Background: Several lines of evidence indicate the altered function of the temporal lobe, including the hippocampus and entorhinal cortex (EC), is associated with schizophrenia. We used single-cell gene expression technologies to assess coordinate changes in the expression of multiple genes, including neuronal signaling and synaptic-related markers in EC layer II stellate neurons.

Methods: We used a single-neuron microdissection technique coupled with linear antisense RNA amplification and high density/candidate gene arrays to assess coordinate changes in gene expression. The expression and relative abundance of more than 18000 messenger RNAs were assessed from EC layer II stellate neurons from postmortem samples of schizophrenic and age-matched control brains. Results of this initial screen were used to perform a more specific secondary messenger RNA screen for each subject.

Results: Data disclosed marked differences in expression of various G-protein–coupled receptor-signaling transcripts, glutamate receptor subunits, synaptic proteins, and other transcripts. Results of secondary screening showed significant decreases in levels of G-protein subunit iα1, glutamate receptor 3, N-methyl-D-aspartate receptor 1, synaptophysin, and sensory nerve action potentials 23 and 25 in the stellate neurons of schizophrenic patients. We observed down-regulation of phospholemman (a phosphoprotein associated with anion channel formation) messenger RNA and protein levels in layer II/III stellate neurons in the population with schizophrenia.

Conclusions: These results provide a preliminary expression profile of schizophrenia in defined neuronal populations. Understanding the coordinated involvement of multiple genes in human disease provides insight into the molecular basis of the disease and offers new targets for pharmacotherapeutic intervention.

Arch Gen Psychiatry. 2002;59:631-640
SUBJECTS AND METHODS

SUBJECTS

Brains from 8 patients who underwent long-term hospitalization for schizophrenia and 9 age-matched neurologically normal controls were used. Postmortem brain tissue from schizophrenic patients was obtained from the established brain collection of the Mental Health Clinical Research Center on Schizophrenia at the University of Pennsylvania, Philadelphia (Table 1). Control tissue was obtained via the Center for Neurodegenerative Disease Research at the University of Pennsylvania. Controls had no history of neurological or major psychiatric illness. We performed gross and microscopic diagnostic neuropathologic examinations, which included examination of multiple cortical and subcortical regions, in all cases, and no neuropathologic abnormalities relevant to mental status were found. Schizophrenic subjects were elderly, “poor-outcome” patients who were participants in clinical-pathologic studies at the University of Pennsylvania School of Medicine in collaboration with 8 state hospitals in eastern and central Pennsylvania. All patients were prospectively accrued, underwent clinical interviews and assessments, and were diagnosed according to DSM-IV criteria1 by research psychiatrists of the Mental Health Clinical Research Center.2 In general, clinical features included prominent negative symptoms, relatively mild positive symptoms, moderate to severe cognitive dysfunction, and impairments in basic self-care activities that warranted the long-term hospitalization of these patients. Antipsychotic treatment was calculated as mean daily chlorpromazine equivalents from dose intervals ranging from no greater than 72 hours, 1 month, and 1 year before death.

IMMUNOCYTOCHEMISTRY

Tissue blocks, which included the middle portion of the EC, were dissected from the temporal lobe at autopsy, fixed in a solution of 70% ethanol/150mM sodium chloride, embedded in paraffin, and cut in 6-mm sections as described previously.3 A section from each individual was stained with acridine orange to verify the presence of nucleic acids in the tissue.4 To identify individual neurons for subsequent single-cell analysis, we performed immunocytochemistry for the sections using a monoclonal antibody to nonphosphorylated neurofilament (RmdO20).5 The antibody was labeled by means of the avidin-biotin method (ABC Vectastain; Vector Laboratories, Burlingame, Calif) and visualized by means of 3,3’-diamino benzidine.

SINGLE-CELL GENE EXPRESSION

After immunolabeling, an oligo(dt)-T7 primer/promoter (AAACGACGGCCCGAGTTGATAACGACTCCTATAGGCGC[T]24 was hybridized to poly A m mRNA overnight in a solution consisting of 50% formamide/5x silver sulfadiazine and chlorhexidine (SSC) at 25°C. Complementary DNA was synthesized directly on the tissue sections (in situ transcription) using avian myeloblastosis virus reverse transcriptase (0.5 U/µL) (Seikagaku America, Falls church, Mass) in Tris buffer containing 6mM magnesium chloride, 120mM potassium chloride, 7mM dithiothreitol, 250µM each of deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxyguanosine triphosphate, and thymidine triphosphate, and 0.12 U/µl of RNAsin.6 Sections were incubated at 37°C for 90 minutes. Next, sections were washed twice in 2X SSC, 28°C for 5 minutes, and stored at 4°C in 0.5X SSC up to 72 hours. After in situ transcription, layer II stellate neurons were dissected using a micropipette attached to a micromanipulator under a high-power objective field (original magnification X40). Contents were collected in the pipette and emptied into 1.5-mL microcentrifuge tubes for second-strand cDNA synthesis and subsequent antisense RNA (aRNA) amplification. The amplification and reamplification procedures are described in detail elsewhere.7,8 Samples were pooled immediately before second-round amplification. We incorporated phosphorus 33-labeled cytidine triphosphate in the pooled sample probes destined for hybridization of human gene discovery arrays (GDA; Genome Systems, Inc, St Louis, Mo). The RNA samples were pooled for each subject and labeled with phosphorus 32 cytidine triphosphate for candidate array hybridization. Under optimal conditions, the first round of aRNA amplification results in an approximately 1000-fold yield and an approximately 104-fold yield after 2 rounds. The aRNA procedure is a linear amplification process with minimal change in the relative abundance of the mRNA population in the native state of the neuron. Messenger RNA can be reliably amplified from small amounts of fixed tissue, including individual neurons and neuronal processes.9,10

For initial screening of the GDAs (>18000 genes), aRNA from 6 neurons from each of 4 schizophrenic indicating decreased synaptic density in this region and other possible alterations in synaptic circuitry. In addition, catecholaminergic and glutamatergic signaling abnormalities have been reported in the temporal lobe of schizophrenic patients, suggesting alterations in the structure and connectivity of this region.11,12

Within the temporal lobe, EC layer II stellate neurons exhibit alterations, including aberrant cytoarchitectural arrangement,13,14 smaller neuron size with normal neuron density,15,16 and decreased expression of the microtubule-associated protein 2.17 The EC layer II stellate neurons constitute an integral component of the conduit through which information flows to the hippocampus, which helps to regulate cortical-hippocampal-subcortical interac-
patients and 4 controls were pooled (eg, 24 neurons per condition for each array) before the second round of amplification. Tissue from the schizophrenic patients was selected on the basis that none had been treated with antipsychotic medication for at least 1 year before death.

CONSTRUCTION OF CANDIDATE ARRAYS

Candidate arrays were prepared on nylon membranes containing, but not limited to, dopamine receptors (eg, D1, D2, D3, D4, and D5), G-protein subunits (α1, α2, αs, αz, αq, β, γ1, and γ2), transcription factors (CREB, CREB2, CREM, junB, and junD c-fos), glutamate receptor mRNAs (AMPA [GluR1-4], kainate [GluR5-7], and N-methyl-D-aspartate receptor 1 [NMDA R1]), and synaptic proteins (α-synuclein, synaptophysin 1 and 2, synaptobrevin, synaptotagmin 2, synaptotygrin 1a and 3, synaptic vesicle-associated protein [SNAP] 23 and 25, postsynaptic density 95, and synaptotagmin VII). Inserts were amplified in 96-well plates using polymerase chain reaction analysis with M13 forward and reverse primers under the following conditions: 95°C for 30 seconds, 32°C for 45 seconds, and 72°C for 2 minutes (40 cycles of this combination); and 72°C for 10 minutes (1 cycle). After polymerase chain reaction analysis, aliquots underwent electrophoresis on a 1% agarose gel (1 × Tris-borate-EDTA pH 8.0 and 0.05% ethidium bromide) at 5 V/cm, and the polymerase chain reaction band size was verified. Gel images were captured by means of a digital camera and archived on a computer. We spotted 250 ng of each amplified insert on a nylon transfer membrane (HyBond XL; Amersham Pharmacia Biotech, Minneapolis, Minn) using a 96-well dot-blot apparatus (MiniBlot; Schleicher & Schuell, Inc, Baltimore, Md). The DNA was crosslinked to the membrane by means of UV radiation.

GDA AND CANDIDATE ARRAY HYBRIDIZATION

Arrays were hybridized for 24 hours at 44°C in a rotisserie hybridization oven (Hybaid, Boston, Mass) with the following solution: 50% formamide, 5 × SSC, 50% Denhardt solution, 0.1% sodium dodecyl sulfate (SDS), 200 ng of sheared salmon sperm, and 1.0 mM sodium pyrophosphate. After hybridization, membranes were washed sequentially with solutions consisting of 2 × SSC/0.1% SDS, 0.5 × SSC/0.1% SDS, and 0.1 × SSC/0.1% SDS for 20 minutes each at 44°C. We detected labeled hybridized products using phosphoimager cassettes, and we analyzed hybridization signal intensities using ImageQuant software (Amersham Pharmacia/Molecular Dynamics, Menlo Park, Calif).

DATA ANALYSIS

The specific signal (minus background) of probe bound to each clone is expressed as a ratio of the total hybridization intensity of the array, thereby minimizing variations due to differences in the specific activity of the probe and the absolute quantity of probe present.23 Differential expression of greater than 2-fold is accepted as above background and relevant for further examination. Two-fold changes are considered a conservative limit. Data from the candidate gene arrays were analyzed by t test, and the null hypothesis was rejected when P < .05.

RELATIONAL DATABASE

Data were imported into the RNA Abundance Database, an Oracle relational database developed at the University of Pennsylvania. The RNA Abundance Database is designed to capture information on RNA abundance assays for any type of high-throughput gene expression experiment, including microarrays, macroarrays, and serial analysis of gene expression tags. For each experiment, hybridization signal intensity for each data point was expressed as a percentage of the total intensity on the array. This enabled comparison of data generated under different conditions and across experimental platforms. To identify genes by functional role or chromosomal location, queries were performed against the database of transcribed sequences (DoTS),34 a component of the Genomics Unified Schema relational database also developed at the University of Pennsylvania and implemented in Oracle. The DoTS contains known and putative transcripts from human and mouse tissues. Each transcript has a consensus sequence assembled by computational analysis of the expressed sequence tag (EST) and known mRNA sequences available in the public databases. These DoTS transcripts were then annotated to assign such things as predicted cellular roles, GO functions, and chromosomal locations.35 The spots in the array experiments can be linked to DoTS transcripts through their respective EST sequences, allowing the assignment of cellular roles of 13 510 and chromosomal location to 11 591 clones. Data sets were selected by means of SQL queries joining the DoTS and RNA Abundance Database, and scattergrams were generated using SigmaPlot software (SPSS Science, Chicago, Ill).

RESULTS

DEMOGRAPHIC DATA

No significant difference was seen between the schizophrenic and control groups in age (t15 = –1.14; P = .27), postmortem interval (t14 = 0.68; P = .51), or brain weight (t14 = –0.45; P = .66), indicating these factors do not contribute to the observed changes in differentially ex-
pressed genes. The schizophrenic group included 5 women and 3 men with an average±SEM age of 83±9.3 years, whereas the age of disease onset was 23.4±3.4 years. The age-matched controls (average±SEM age, 77.7±12.2 years) consisted of 5 women and 4 men.

IMMUNOCYTOCHEMISTRY AND RNA RECOVERY

Examination of tissue sections after immunolabeling with RmdO20 disclosed a distinct laminar pattern of immunoreactivity that was confined to the somatodendritic region of neurons in layers II/III and V of the EC (Figure 1A-B). No distinct differences in the intensity or pattern were apparent between the groups. Immunostaining was used to delineate layer II stellate neurons for microdissection (Figure 1C-D). As in previous studies, no apparent difference was seen in mRNA recovery between the groups.45

GENE EXPRESSION

The GDA format contained 18240 genes, of which 2574 (14%) were up-regulated more than 2-fold in the schizophrenic group and 1565 (9%) were down-regulated. In addition, we examined a subgroup of transcripts that encode proteins (13910 mRNAs) of known function and are designated as all cell roles in the Institute for Genomic Research database. Changes in gene expression were assessed by the degree of differential expression in specific functional families encoding all cell roles, receptors (292 clones), intracellular transducers (169 clones, including G proteins and second-messenger systems), and extracellular matrix proteins (199 clones, including synaptic proteins). The subsets were selected because of the key role members of each of these families play in cellular functioning, not necessarily because of their significance in schizophrenia. For convenience, differences in mRNA levels for these categories are shown in Figure 2. The complete expression profiles generated in this study are available in Excel format via e-mail (available at: eberwine@pharm.med.upenn.edu).

RECEPTORS

The absence of dopamine receptor subtypes on the GDA arrays necessitated inclusion of these clones of the custom-designed candidate arrays. No significant difference was seen in mRNA abundance for D1, D2, D4, or D5 receptor subunits between the schizophrenic and control groups (Figure 3A). Analysis showed an up-regulation in serotonin receptor mRNA (+3.0-fold). The H2-adrenergic receptor mRNA was down-regulated 2.1-fold, a finding consistent with the reduced H2-adrenergic receptor binding in the limbic system of the schizophrenic brain.46 Secondary screening of G-protein subunits that couple to monoamine receptors disclosed a significant decrease in Gi1 subunit mRNA (t15=2.37; P=.03) and a significant increase in Gq2 subunit mRNA (t15=−2431; P=.03) levels in schizophrenic patients (Figure 3).

Several groups have reported alterations in γ-aminobutyric acid and glutamate receptor protein and mRNA subunits in the schizophrenic brain.26,27,47-55 Consistent with these findings, we found a 4.2-fold increase in γ-aminobutyric acid A1 subunit mRNA in schizophrenic patients. No significant differences were detected in NMDA R2A, GluR1, GluR2, or GluR6 on the GDA arrays, and none were detected for GluR1, GluR4, and GluR5 on the custom-designed arrays. However, GluR3 was found to be significantly down-regulated on the GDA arrays (−2.2-

<table>
<thead>
<tr>
<th>Table 1. Case Information*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>Schizophrenic Patients</td>
</tr>
<tr>
<td>S1</td>
</tr>
<tr>
<td>S2</td>
</tr>
<tr>
<td>S3</td>
</tr>
<tr>
<td>S4</td>
</tr>
<tr>
<td>S5</td>
</tr>
<tr>
<td>S6</td>
</tr>
<tr>
<td>S7</td>
</tr>
<tr>
<td>S8</td>
</tr>
<tr>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Nonpsychiatric controls</td>
</tr>
<tr>
<td>C1</td>
</tr>
<tr>
<td>C2</td>
</tr>
<tr>
<td>C3</td>
</tr>
<tr>
<td>C4</td>
</tr>
<tr>
<td>C5</td>
</tr>
<tr>
<td>C6</td>
</tr>
<tr>
<td>C7</td>
</tr>
<tr>
<td>C8</td>
</tr>
<tr>
<td>C9</td>
</tr>
<tr>
<td>Mean (SD)</td>
</tr>
</tbody>
</table>

*PMI indicates postmortem interval; NA, not available; and ellipses, not applicable.
†Indicates daily antipsychotic medication dose 1 month before death, expressed in chlorpromazine equivalents.
‡Includes 3 male and 5 female patients.
§Includes 3 male and 6 female controls.

(ORIGINAL) ARCH GEN PSYCHIATRY/VOL 59, JULY 2002 WWW.ARCHGENPSYCHIATRY.COM
©2002 American Medical Association. All rights reserved.
fold) and confirmed using the custom-designed arrays ($t_{15}=2.18; P=.045$). In addition, NMDA R1 was significantly down-regulated in the schizophrenic group ($t_{15}=2.55; P=.02$; Figure 4B) using the custom arrays.

Cholinergic dysfunction has also been implicated in schizophrenia, including decreased nicotinic receptor binding in the hippocampus$^{56}$ and demonstration of a di-nucleotide polymorphism at chromosome 15q13-14, the site of the $\alpha7$ subunit of the nicotinic receptor.$^{57}$ Extending these reports, we found a 2.7-fold increase in expression of the $\alpha7$ subunit mRNA in EC stellate neurons in our schizophrenic population.

GENES ASSOCIATED WITH SYNAPTIC PROTEINS

Several synaptic protein mRNAs were differentially regulated between the schizophrenic and control groups, including down-regulation in schizophrenia of $\gamma$-adaptin ($-5.5$-fold), synaptic vesicle amine transporter ($-3.5$-fold), synaptotagmin I ($-3.1$-fold), synaptotagmin IV ($-2.5$-fold), and SNAP 25 ($-4.4$-fold). An example of the differential hybridization intensity for SNAP 25 on a GDA filter is provided in Figure 4. In addition, syntaxin mRNA was up-regulated ($+4.4$-fold) in schizophrenic patients. Assessment of several synaptic protein mRNAs using the candidate arrays showed significant decreases in synaptophysin ($t_{15}=2.22; P=.04$), SNAP 23 ($t_{15}=2.94; P=.01$), and SNAP 25 ($t_{15}=2.09; P=.055$) mRNA levels in schizophrenic patients (Figure 3B).

PHOSPHOLEMMAN EXPRESSION

Differential hybridization to the cDNAs encoding several ESTs was noted, in addition to genes of known function. One of the most highly regulated ESTs corresponded to phospholemman (PLM), a phosphoprotein involved in the formation and/or regulation of a chloride anion channel. Expression levels of PLM mRNA in single EC stellate neurons were lower in schizophrenic brains than in those of matched controls ($-4.5$-fold). We were unable to perform secondary screening on PLM mRNA abundance because of the lack of clone in the human clone set (Emory Functional Genomics Facility, Atlanta, Ga). To determine whether PLM protein was
present in layer II/III stellate neurons, a polyclonal antibody against PLM was used to stain sections adjacent to those used for neuronal dissection and mRNA analysis. Immunoreactivity of PLM was detected in 2 distinct cellular compartments in the human brains (Figure 5A-B), and a similar distribution was observed in rat brains (data not shown). Diffuse cytoplasmic PLM immunoreactivity was detected within the perikarya of EC stellate neurons and neocortical pyramidal cells, and punctate PLM immunoreactivity was found in preterminal axons and terminal fields throughout the hippocampal formation. Perforant pathway labeling was particularly distinct (Figure 5C). Semiquantitative assessment (by experimenters who were blind to the diagnosis) of the 24 cases disclosed differences in PLM immunoreactivity within the perikarya of layer II EC stellate neurons. Specifically, perikaryal PLM immunoreactivity in EC stellate neurons was consistently less intense in the schizophrenic brains than in the normal control brains (Figure 5A-B). No obvious differences were observed in the intense axonal/terminal labeling of the perforant pathway axons that traverse the subicular complex and terminate within the dentate gyrus.

GENES ASSOCIATED WITH REPORTED SCHIZOPHRENIA LINKAGE SITES

Approximately 25% of the genes in the public databases have been mapped to chromosomal loci. We have used this information to examine the relative abundances of various mRNAs whose genes map to presumed schizophrenia linkage sites (Table 2). In this analysis, it is clear that the abundances of most of these mRNAs remain relatively unchanged within these regions, whereas some show dramatic differences. Individually, these particular mRNAs are unlikely to be key causative factors of schizophrenia, yet small changes in multiple genes spanning these different chromosomal sites may indeed result in an altered cellular physiological presentation and contribute to the schizophrenic phenotype. Since only a small fraction of the ESTs have been mapped to chromosomal sites, we are continuing to map mRNAs whose abundance is significantly different in schizophrenia. The present expression analysis examines only the relative prevalence of mRNAs; we have not examined potential genetic polymorphisms that may be associated with these specific genes and result in the observed difference in mRNA abundance in schizophrenic patients relative to controls.

RESULTS

Results from the present study have identified several possible mechanisms of neuronal dysfunction that may underlie aspects of schizophrenia. One such mechanism involves vesicular proteins in synaptic function. Levels of glutamate receptors, G-protein subunits, and synaptic proteins were measured in the entorhinal cortex (EC) layer II stellate neurons in schizophrenic brain for all cell roles (A), receptors (B), intracellular transducers (C), and extracellular matrix proteins (D). Normalized expression of values in age-matched controls and schizophrenic samples are plotted. Red line indicates no change; blue lines, 2-fold up- or down-regulation; and black lines, 5-fold up- or down-regulation.
of mRNAs encoding synaptic vesicle proteins (synaptophysin and synaptotagmin I and IV) and synaptic plasma membrane proteins (SNAP 23 and SNAP 25) were found to be significantly decreased in EC layer II stellate neurons of schizophrenic patients, whereas another plasma membrane protein syntaxin was up-regulated greater than 4-fold. The proteins encoded by these mRNAs serve different functions at different functional steps in the synaptic vesicle cycle, and it is reasonable to conclude that alterations in the levels of the proteins encoded by these mRNAs may lead to decreased neurotransmitter release from the layer II stellate neurons. For exocytosis to occur, a trimeric core complex must be formed consisting of 2 synaptic plasma membrane proteins and 1 synaptic vesicle protein.58 Decreased levels of SNAP 25 may prevent the establishment of the anchor complex for vesicular docking to the plasma membrane. Furthermore, decreased levels of synaptotagmin I and IV, which bind the calcium2+ ion and possibly serve as a sensor for exocytosis,59 indicate another potential means of decreased neurotransmitter release. These findings are paralleled by studies demonstrating decreased synaptic vesicle protein mRNA and protein levels in the temporal cortex18,20-24 and other brain regions.22,35,60-64 The altered expression of SNAP 25 and syntaxin are not likely due to long-term antipsychotic treatment, since long-term haloperidol decanoate administration in rodents does not affect SNAP 25 mRNA expression and decreases syntaxin and synaptophysin mRNA expression.55,66 However, the observed decreases in synaptotagmin I and IV mRNA levels in schizophrenic patients may be attributable in part to the treatment history, since long-term haloperidol administration also decreases synaptotagmin mRNA levels,65 although extrapolations of these data to humans should be made with caution.

Results of high-density array analysis indicate down-regulation of β2-adrenergic receptor mRNAs46 and up-regulation of the γ-aminobutyric acid Aε12-35 subunit and serotonin receptor mRNA, findings that are consistent with those of previous studies. No significant differences were observed for the dopamine receptor subtype mRNAs in the present study. However, Gia1 and Gγ2 subunit mRNA levels were significantly reduced and elevated, respectively, in the schizophrenic population, a finding consistent with Gia immunoreactivity in the temporal cortex of schizophrenic patients.67 Glutamatergic dysfunction is yet another possible mechanism underlying the neuropathophysiology of schizophrenia, spe-
specifically, the gene and protein expression of the ionotropic subtypes in human postmortem tissue. For example, previous studies have demonstrated decreased expression of GluR1 and GluR2 mRNAs in hippocampal subfields and NMDA R1 mRNA in the temporal cortex. Extending these findings, NMDA R1 and GluR3 were down-regulated in EC layer II stellate neurons in the present study. Dysregulation in ionotropic glutamate receptors may have profound downstream effects, including alterations in excitatory neurotransmission and subsequent cognitive and behavioral sequelae believed to be driven by glutamatergic circuitry.

In addition to genes known to be involved in synaptic function, array analysis led to the identification of PLM mRNA in the EC layer II stellate neurons. Phospholemman is a phosphoprotein involved in the formation and/or regulation of a chloride anion channel enriched in cardiac and skeletal muscle, although results of Northern blot analysis have demonstrated moderate mRNA expression in total brain homogenates. Perikaryal PLM immunoreactivity in EC stellate neurons was consistently less intense in the schizophrenic brains than in the normal control brains. The observed EC staining pattern is not selective to our brain collection population; it was replicated in EC tissue sections from 2 schizophrenic patients obtained from the Stanley Foundation Brain Bank, Bethesda, Md. Further studies are warranted to characterize the neuroanatomical distribution of PLM, to delineate the functional role of this protein in the brain, and to further assess the contribution of PLM down-regulation in schizophrenia.

Chromosomal mapping of genes that are altered in schizophrenia may provide insight into how the chromosomal abnormality is manifested in the symptomatology of schizophrenia. These genes may map directly a chromosomal breakage, but more likely are adjacent genes whose regulation is affected in schizophrenia. Such regulatory differences may be associated with polymorphisms in the promoter regions of these genes that, in turn, alter transcription rates leading to changes in mRNA abundance. Individual mRNAs are unlikely to be singular causal factors for schizophrenia. However, small changes in multiple genes spanning these different chromosomal loci may result in an altered cellular physiology, thus contributing to the schizophrenic phenotype.

A common confound in using human tissue for neuropathophysiological examinations lies in the clinical diagnosis of the individual patient. In the present study, the use of a prospective collection of brains from subjects who underwent clinical assessment during life obviates this problem. Since the pharmacological course of treatment for schizophrenia may influence gene expression, initial screening of arrays used brain tissue from patients who had not received antipsychotic medication for at least 1 year before death, followed by secondary screening of all subjects in the sample population regardless of medication history. The observed consistency in these hybridization patterns is likely due to the long-term treatment histories of all subjects in the study. Nevertheless, the influence of medication exposure on gene expression cannot be discounted. The postmortem interval was similar to or less than that of other studies and is unlikely to grossly influence the molecular analysis presented herein. Nonetheless, the utility of an expression profile specific for schizophrenia can be envisioned. For example, differentially expressed transcripts could serve as an additional postmortem diagnostic tool. Application of similar technologies to generate peripheral markers may enable more rapid diagnosis and pharmacological intervention tailored to the patient’s specific symptoms. In addition, confirmation of corresponding changes at the protein level may provide novel targets for drug discovery and/or a refinement of existent pharmacotherapies. In the future, disease-related transcripts might also be targets for gene therapy interventions.

Submitted for publication January 21, 2000; final revision received September 26, 2001; accepted October 18, 2001.

From the Departments of Pharmacology and Psychiatry and Behavioral Sciences, Yerkes Regional Primate Research Center, Neuroscience Division, Emory University School of Medicine, Atlanta, Ga (Dr Hemby); the Dementia Research Program, Department of Psychiatry, Nathan Kline Institute, New York University School of Medicine,
Orangeburg (Dr Ginsberg); and the Center for Bioinformatics (Dr Brunk) and the Departments of Psychiatry (Drs Arnold and Eberwine), Neurology (Drs Arnold and Eberwine), Pharmacology (Dr Eberwine), and Pathology and Laboratory Medicine (Dr Trojanowski), University of Pennsylvania School of Medicine, Philadelphia. Dr Hemby is a consultant for Solvay Pharmaceuticals, Utrecht, the Netherlands. Dr Eberwine is on the Scientific Advisory Board of Incyte Pharmaceuticals, Sunnyvale, Calif, which owns Genome Systems. Drs Eberwine and Trojanowski are Founding Scientists, consultants and stockholders for Layton Bioscience, Inc, Sunnyvale, which has licensed the aRNA amplification and in situ transcription methods.

This study was supported by the Walter Sondheim Katz National Alliance for Research on Schizophrenia and Depression (NARSAD) Young Investigator Award (Great Neck, NY) and a National Alliance for Autism Research Award (Princeton, NJ) (Dr Hemby), a NARSAD Distinguished Investigator Award (Dr Eberwine), grants AG10124 and AG09215 from the National Institute on Aging (Bethesda, MD) (Dr Trojanowski), and grants MH55199 (Dr Arnold) and MH43880 (Drs Trojanowski and Arnold) from the National Institute of Mental Health (Bethesda).

The Functional Genomics Facility of the Emory University School of Medicine, Atlanta, Ga, provided the cDNA clones for secondary screening, and the Stanley Foundation, Bethesda, kindly provided the phospholemman antibody. The authors thank the staff of the Mental Health Clinical Research Center on Schizophrenia and the Department of Pathology and Laboratory Medicine of the University of Pennsylvania for their assistance in case accrual and evaluation.

Corresponding author and reprints: James H. Eberwine, PhD, Department of Pharmacology, University of Pennsylvania, 3620 Hamilton Walk, Philadelphia, PA 19104.

REFERENCES


