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Microarray analysis of gene expression in the prefrontal cortex in schizophrenia: a preliminary study

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Abstract

Microarray studies can be used to examine expression levels for large numbers of genes simultaneously and may be applied to identify genes involved in schizophrenia. A microarray with 1127 brain-relevant genes was used to screen relative gene expression in the dorsolateral prefrontal cortex (DLPFC) from three pools of patients with schizophrenia ($n=15$) and three matched control pools ($n=15$). Pooling of tissue samples was employed as a strategy to detect changes in gene expression that are consistently found across individual cases of schizophrenia. Differences in gene expression were examined by z -ratios in addition to traditional normalized ratios. Three genes that showed consistently decreased expression in schizophrenia by both z -ratio differences and decreased normalized numerical ratios were identified. These were histidine triad nucleotide-binding protein (HINT), ubiquitin conjugating enzyme E2N (UBE2N) and glutamate receptor, ionotropic, AMPA 2 (GRIA2). Moreover, HINT gene expression was decreased to a similar degree in a prior study. In addition, a decrease in AMPA receptor expression is consistent with a decrease in glutamate synaptic function. These results are subject to limitations based on variations inherent to human subjects and tissue samples, possible effects of neuroleptic treatment, and the requirement for verification using independent techniques.

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Keywords: Microarray; Schizophrenia; Genomics; Gene expression; Postmortem; Prefrontal cortex

1. Introduction

Differences in gene expression may underlie the pathophysiology of schizophrenia. A number of indi-

vidual molecular differences between patients with schizophrenia and controls have been identified by various techniques (Harrison, 1999). Massive parallel screening techniques such as the microarray methodology employed in this study and other investigations of schizophrenia (Hakak et al., 2001; Mirmics et al., 2000, 2001; Vawter et al., 2001) have been used to simultaneously examine large numbers of genes for differential expression. Through the use of microarrays, novel candidate genes can hopefully be iden-

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tified, thereby rapidly advancing the field of schizophrenia research.

We adopted an initial approach of using a specialized cDNA microarray (Whitney et al., 1999) comprised of 1127 human genes selected for relevance to brain function, i.e., a “neuroarray” (Barrett et al., 2001; Vawter et al., 2001), thereby enabling screening of interesting genes with relatively small pools of total RNA. In the present study, each of three pools of patients with schizophrenia were matched to a corresponding pool of control subjects. Neuroarrays were probed with total RNA extracted from the dorsolateral prefrontal cortex (PFC) [DLPFC; Brodmann area (BA) 9 and 46].

2. Methods

2.1. Neuroarray

The details of the development of the NIA-Neuroarray (URL <http://www.grc.nia.nih.gov/branches/rrb/dna.htm>) are being reported elsewhere (Barrett et al., 2001; Vawter et al., 2001). Briefly, a 15,000-human cDNA clone set of IMAGE Consortium clones (<http://image.llnl.gov/>) available from Research Genetics (Huntsville, AL) was sorted to obtain 1127 genes relevant to brain function and neuropsychiatric disorders. cDNAs were obtained from the selected bacterial clones by PCR amplification with universal primers, followed by spotting of ~5–10 nl of reaction product/300- μ m spot, in duplicate, onto Nytran + Supercharge membranes (Schleicher & Schuell, Keene, NH) with a Genetic Microsystems 417 printer (Woburn, MA).

2.2. Samples and RNA extraction

Samples of DLPFC were from the Section on Neuropathology of the Clinical Brain Disorders Branch, NIMH Intramural Research Program. Total RNA was extracted using a modified version of the TRIZOL[®] method (Life Technologies, Grand Island, NY). Frozen tissue samples were homogenized for 45 s using a Brinkmann Polytron[™] tissue homogenizer at 4 °C,

moderate speed, in 1 ml TRIZOL[®] Reagent per 30–50 mg of tissue. The homogenate was maintained at 4 °C for a further 1 min after which the homogenizing process was repeated, and the manufacturers’ protocol was followed. RNA pellets were dried for 15 min and resuspended in 0.1% diethylpyrocarbonate-treated dH₂O. Samples were stored at –80 °C. The RNA samples were found to be of high quality by spectrophotometry and agarose gel electrophoresis. Pools of total RNA (20 μ g) were formed by using equal amounts of total RNA from each individual matching, where possible, for age, gender, PMI, freezer time and pH (Table 1). In addition, data from a fourth pool of PFC tissue from a previous study (Vawter et al., 2001) were compared to the current results.

2.3. RNA labeling and hybridization

Total RNA was radioactively labeled with [³³P]-dCTP, for details see: <http://www.grc.nia.nih.gov/branches/rrb/dna.htm>. Briefly, total RNA (20 μ g) was reverse-transcribed to cDNA with reverse-transcriptase enzyme in the presence of [³³P]-dCTP. The [³³P]-dCTP-cDNA was purified through a spin column by size separation (BioSpin, Bio-Rad, CA) from [³³P]-P-dCTP and the heat denatured probe (~5 \times 10⁶ cpm) was diluted in 4 ml of Microhyb solution (RG) and hybridized to the neuroarray for 16–18 h at 50 °C with rotation. The neuroarrays were rinsed once with 2 \times SSC at room temperature, and then washed twice with agitation at 45 °C for 10 min in 45 ml of 2 \times SSC and 0.1% SDS buffer (Stillman and Tonkinson, 2001). The neuroarray was placed under Saran wrap and exposed to a low-energy phosphor screen (Molecular Dynamics, Sunnyvale, CA) for 1–5 days and scanned in a Phosphorimager 860 (Molecular Dynamics) at 50 μ m resolution.

2.4. Statistical analysis and calculations of z-ratios

Data were analyzed using a z-score normalization method. This method involves calculating a distribution of z-scores for all genes in each array, and employing the differences of the z-scores between the

Note to Table 1:

Gender: M, male; F, female. PMI, postmortem interval. FT, freezing time of tissue. CUS, chronic undifferentiated schizophrenia ($n=11$). CDS, chronic disorganized schizophrenia ($n=3$). CPS, chronic paranoid schizophrenia ($n=1$). TD, tardive dyskinesias ($n=3$). ETOH, alcohol abuse or SA, substance abuse ($n=4$).

Table 1
Demographic and postmortem data for schizophrenia patients and normal control subjects

Subject #	Age (years)	Sex	Race	PMI (h)	FT (months)	Brain pH	Age of onset	Cause of death	Manner of death	DSM-IV diagnosis
<i>Pool 1—control</i>										
822	26	M	C	14.5	92	6.08		Arteriosclerotic cardiovascular disease (ASCVD)	Natural	
374	58	F	AA	26.5	203	6.54		Cardiac arrest	Natural	
928	24	M	AA	12.5	35	6.59		Fibrinous pericarditis	Natural	
83	68	M	AA	25.0	169	6.57		ASCVD	Natural	
1099	67	F	AA	34.0	49	6.69		Dilated cardiomyopathy	Natural	
Mean	49			22.5	110	6.49				
<i>Pool 1—schizophrenia</i>										
42	48	M	AA	48.5	159	6.42	22	ASCVD	Natural	CUS/ETOH
838	31	M	C	15.5	51	6.46	17	Undetermined	Natural	CDS
766	64	F	AA	20.5	98	6.48	19	Asphyxia secondary to aspiration	Accident	CUS/TD
802	67	F	AA	39.5	95	6.63	30	Respiratory failure	Natural	CUS
884	35	M	AA	79.5	87	6.70	22	Acute pulmonary embolism after fall	Suicide	CDS
Mean	49			40.7	98	6.54	22			
<i>Pool 2—control</i>										
52	34	M	AA	37.0	162	6.60		ASCVD	Natural	
644	60	F	C	10.0	122	6.20		ASCVD	Natural	
639	55	M	AA	10.5	123	6.00		Acute myocardial infarction due to ASCVD	Natural	
924	42	M	AA	40.0	74	6.63		Acute asthmatic attack	Natural	
977	56	M	AA	34.0	65	6.09		Pulmonary embolism	Natural	
Mean	49			26.3	109	6.34				
<i>Pool 2—schizophrenia</i>										
37	71	F	C	47.5	158	6.41	15	ASCVD	Natural	CUS/TD
130	46	M	AA	25.0	173	6.73	37	Fall	Suicide	CUS
938	61	F	AA	21.5	72	6.74	34	Asphyxia, aspiration of food	Accident	CUS
878	30	M	AA	72.5	83	6.32	16	Bilateral pneumonia, cardiac hypertrophy	Natural	CUS/SA
978	38	M	AA	62.0	65	6.50	16	Acute peritonitis, swallowed battery	Accident	CDS/ETOH/SA
Mean	49			45.7	110	6.54	24			
<i>Pool 3—control</i>										
536	46	F	AA	21.5	124	5.93		Dilated cardiomyopathy	Natural	
642	48	F	AA	18.5	122	6.06		Pulmonary artery thrombosis	Natural	
517	40	M	C	48.5	139	6.44		ASCVD	Natural	
1069	59	F	AA	37.0	49	6.57		Cirrhosis of liver	Natural	
910	52	F	AA	12.0	77	6.67		Ruptured aorta	Natural	
Mean	49			27.5	102	6.38				
<i>Pool 3—schizophrenia</i>										
634	48	M	C	15.0	85	6.29	28	Hyponatremia	Natural	CUS/TD
70	36	M	AA	13.0	131	6.56	21	Fall	Suicide	CUS
839	23	M	AA	43.0	89	6.46	22	Anoxia secondary to seizures	Natural	CUS
855	60	F	AA	19.0	87	6.38	40	ASCVD	Natural	CPS
763	75	M	AA	41.5	98	6.29	29	Undetermined	Natural	CUS/ETOH
Mean	48			26.3	98	6.40	28			

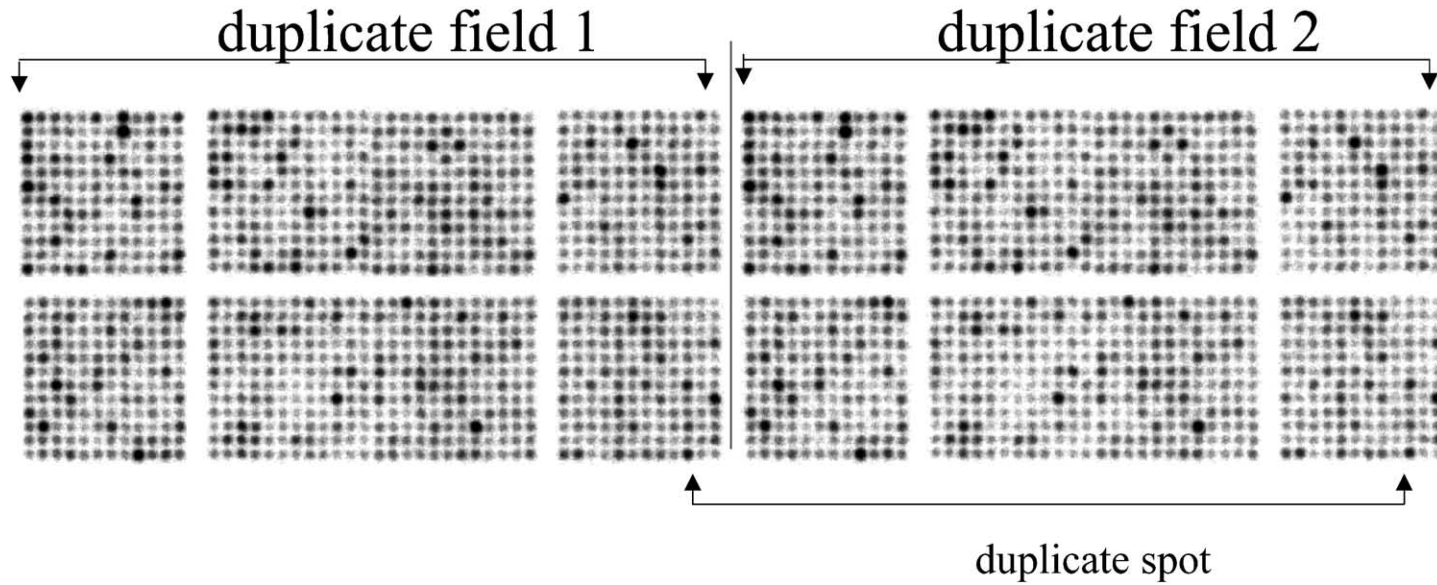


Fig. 1. An example of a neuroarray for one of the DLPFC control pools. Each neuroarray contained 1127 genes arrayed in duplicate as shown. The brighter spots indicate greater amounts of hybridization.

two conditions to search for genes for which expression is changed (for details, cf. Vawter et al., 2001).

In brief, the raw intensity data are transformed by \log_{10} . The mean and standard deviation of the \log_{10} scores were calculated and entered into a z-score normalization formula: Observed gene z-score = (observed gene \log_{10} intensity – mean neuroarray \log_{10} intensity) / (standard deviation neuroarray \log_{10} intensity). Gene expression differences between two neuroarrays, i.e., schizophrenia and control, were calculated by taking the difference between observed gene z-scores. z-Ratios were calculated from the z-score differences using the formula: z-ratio = (z-score difference gene / standard deviation of the z differences distribution). For the present study, 10% of genes showing the greatest decreases or increases in each schizophrenia pool as compared to the corresponding control pool were considered to be of potential interest.

In order to calculate normalized ratios, first, a mean intensity was obtained for all 1128 genes in each neu-

roarray grid. Each neuroarray was then scaled according to the mean intensity of all 1128 genes in the grid divided by the grand mean of all schizophrenia and control neuroarray grids combined. This scaling factor was then applied to each gene in the array to obtain a scaled raw intensity. These scaled raw intensities for the schizophrenia neuroarray grids were then divided by the corresponding genes in the control neuroarray grids to obtain normalized ratio values. Thus, numbers less than one indicate decreased expression in schizophrenia, and numbers greater than one indicate increased expression in schizophrenia.

3. Results

3.1. Array analysis

An example of a neuroarray is shown in Fig. 1. The data were analyzed by both z-ratios and normalized

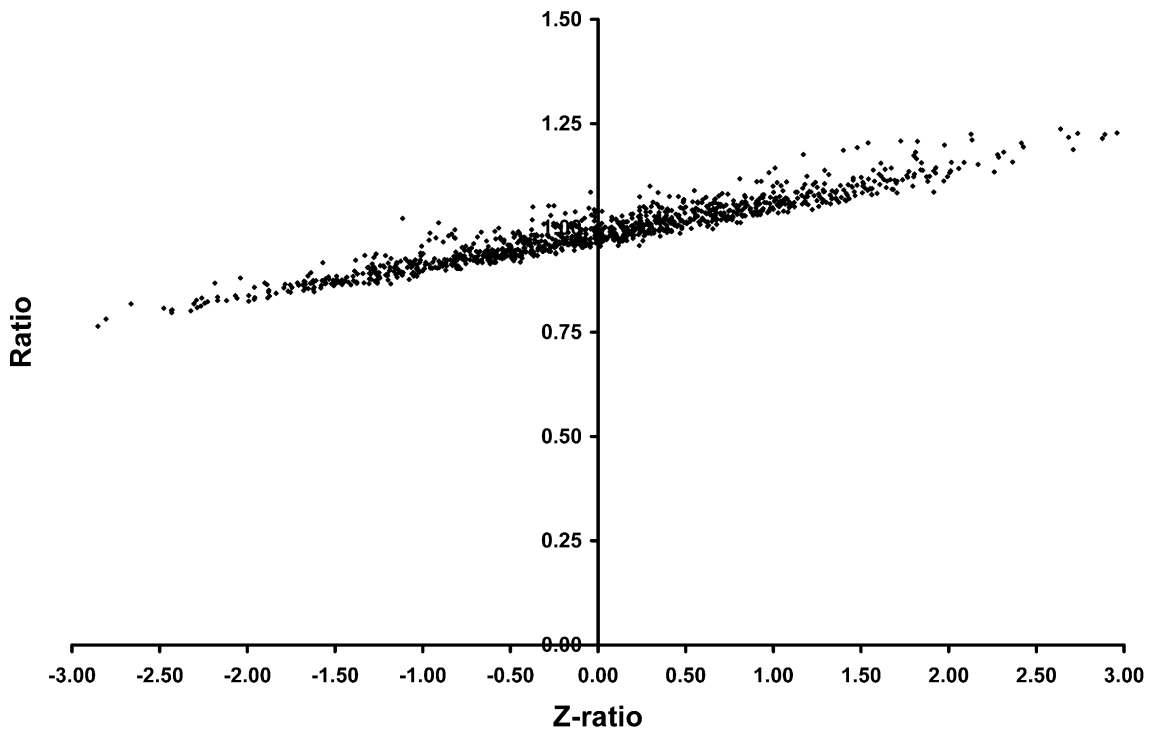


Fig. 2. The x-axis represents the z-ratio for schizophrenia compared to controls showing the average of duplicate neuroarray values. The y-axis represents a traditional ratio of schizophrenia/controls for the average of duplicate values on the Neuroarray. The traditional ratio intensity values were scaled to the grand average of all arrays used in this experiment by multiplying each array by a scaling factor.

intensity ratio. A scatterplot showing the relationship between *z*-ratio and normalized intensity ratios is shown in Fig. 2.

3.2. Current data

There were six differentially expressed genes that met a criterion of being in the 10% of genes that were most increased or 10% most decreased, for all three of the comparisons between pools of patients with schizophrenia and controls. These genes are shown in Table 2.

By chance, one gene would be expected to increase and one gene decrease ($0.1 \times 0.1 \times 0.1 \times 1128$ genes). Using this criterion, therefore, two genes would be expected to have met the present criterion by chance alone.

z-Ratio values were compared to normalized ratios for absolute levels of gene expression. For the genes in Table 2, the absolute changes in gene expression were

relatively small. For the genes with decreased expression in schizophrenia, the changes were on the order of 20% ranging from a ratio of 0.77 ± 0.01 for the HINT gene to 0.82 ± 0.01 for FLJ20258. For those genes showing increased expression, the normalized ratios of expression in schizophrenia to that in controls were from 1.05 for NDUFA4 to 0.97 for TNFRSF6 (Table 3).

3.3. Comparison to PFC (BA9 pool)

We compared the results of the three DLPFC pools that were ran for the current study to a PFC pool also previously ran on the neuroarray (Vawter et al., 2001). For this comparison, the inclusion criterion was relaxed to include all genes that were consistently in the 25% of genes most increased or most decreased for the three DLPFC pool sets in the current study (Table 2).

Table 2

Differential gene expression in schizophrenia: comparisons of three DLPFC subject pools and one PFC pool

Gene name	Gene symbol	GenBank accession number	Chromosome locus	<i>z</i> -ratio ^a				Mean <i>z</i> -ratio ± S.E.M.
				DLPFC1	DLPFC2	DLPFC3	PFC (Stanley)	
<i>Decreased expression</i>								
Histidine triad nucleotide-binding protein *	HINT	T57556	5q31.2	-1.65	-1.78	-1.82	-1.63	-1.72 ± 0.04
Ubiquitin conjugating enzyme E2N *	UBE2N	AA490124	12	-1.38	-2.03	-2.23	-0.34	-1.49 ± 0.42
Glutamate receptor, ionotropic, AMPA2 *	GRIA2	H28734	4q32-q33	-1.30	-1.70	-1.90	-0.62	-1.38 ± 0.28
Mitogen-activated protein kinase 14	MAPK14	AA404479	6p21.3-21.2	-1.56	-1.75	-0.63	-1.21	-1.29 ± 0.24
Serine/threonine kinase 15	STK15	R19158	20q13.2-13.3	-1.04	-1.88	-1.03	-1.13	-1.27 ± 0.20
GABA-A receptor delta subunit	GABRD	H41122	1p36.3	-0.86	-0.70	-1.35	-0.87	-0.95 ± 0.14
Hypothetical protein	FLJ20258	AA464955	19	-0.87	-1.17	-0.99	-0.56	-0.90 ± 0.12
<i>Increased expression</i>								
NADH dehydrogenase/ubiquinone MLRQ subunit *	NDUFA4	AA680322	7	2.89	1.86	1.32	0.35	1.60 ± 0.53
Alpha2, 3-sialyltransferase *	ST3GALVI	N32295	3	1.61	1.93	2.26	-0.32	1.37 ± 0.58
Neuronal apoptosis inhibitor protein *	BIRC1	H21071	5q13.1	1.67	1.34	1.63	-0.26	1.09 ± 0.45
Tumor necrosis factor receptor superfamily, member 6	TNFRSF6	AA293571	10q24.1	0.72	1.50	1.20	0.74	1.04 ± 0.18

“Stanley” indicates a previously reported dataset from the Stanley collection (Vawter et al., 2001). The five genes not marked with an asterisk met a criterion of being in the 25% most increased or decreased for the three DLPFC pool comparisons.

^a Positive numbers indicate increased expression in schizophrenia.

* These genes met a criterion of being in the 10% most increased or most decreased for the three DLPFC pool comparisons.

Table 3
Comparisons of z-ratios to normalized ratios for the three DLPFC pools

Gene name	Normalized ratio \pm S.E.M.	Mean z-ratio	Mean z-score (for control pools)
<i>Decreased expression</i>			
Histidine triad nucleotide-binding protein *	0.77 \pm 0.04	-1.75	1.19
Ubiquitin conjugating enzyme E2N *	0.77 \pm 0.06	-1.88	2.01
Glutamate receptor, ionotropic, AMPA2 *	0.78 \pm 0.05	-1.63	0.71
Mitogen-activated protein kinase 14	0.79 \pm 0.03	-1.31	-0.21
Serine/threonine kinase 15	0.80 \pm 0.04	-1.32	-0.69
GABA-A receptor delta subunit	0.82 \pm 0.03	-0.97	0.13
Hypothetical protein	0.82 \pm 0.01	-1.01	-0.068
<i>Increased expression</i>			
NADH dehydrogenase/ubiquinone MLRQ subunit *	1.05 \pm 0.07	2.02	-0.68
Alpha2,3-sialyltransferase *	1.04 \pm 0.03	1.93	-0.44
Neuronal apoptosis inhibitor protein *	1.00 \pm 0.04	1.55	0.78
Tumor necrosis factor receptor superfamily, member 6	0.97 \pm 0.02	1.14	-1.44

* These genes met a criterion of being in the 10% most increased or most decreased for the three DLPFC pool comparisons.

3.4. Comparison to *Mirmics et al. (2000)*

In order to compare the present data to previously reported changes in gene expression in schizophrenia, genes included in the neuroarray were grouped according to previously published gene classifications from the Incyte GEM platform used by *Mirmics et al. (2000)*. Genes in the categories PSYN (presynaptic), GLUT (glutamate) and GABA (gamma aminobutyric acid) were reported to show decreased expression in schizophrenia. Changes in expression of all 41 genes present in the neuroarray for these three categories, for the three DLPFC pools in the present study, are shown in *Table 4*. Of the eight specific genes identified by *Mirmics et al. (2000)* as being decreased either consistently or occasionally in schizophrenia, five were present in the neuroarray and these are indicated by asterisks in *Table 4*. Within the population of 41 genes shown in *Table 4*, the five genes identified by *Mirmics*

et al. (2000) were ranked among those showing the greatest degree of decreases. The probability that this distribution occurred by chance was small ($U=15$; $p < 0.003$ for comparison of the five genes indicated by asterisks in *Table 4* to the remaining 36 genes by a Mann–Whitney test, grouping all three gene categories). Therefore, the specific genes identified as being decreased in schizophrenia by *Mirmics et al. (2000)* were also decreased in the present study. On the other hand, the mean z-ratios for the entire group of genes in these categories showed a range of values and no overall mean decrease (PSYN = -0.15, GLUT = -0.12 and GABA = -0.09); thus, no overall change in expression of PSYN, GLUT or GABA genes was detected.

4. Discussion

The present data set identified three genes which were within the 10% of genes most decreased for all three matched sets of DLPFC tissue pools by z-ratios that also showed changes in expression by normalized ratios. For the genes showing increased expression in schizophrenia, the numerical ratios of expression values indicated that the absolute magnitude of the increases in expression were negligible. The genes lower in schizophrenia were: histidine triad nucleotide-binding protein (HINT), ubiquitin conjugating enzyme E2N (UBE2N) and glutamate receptor, ionotropic, AMPA 2 (GRIA2).

The pooling of subjects employed in the present study has a potential advantage for microarray studies of helping to mask noise and decreasing the number of spurious findings. On the other hand, individual variations in gene expression abnormalities in schizophrenia would not be detected by the pooling strategy employed.

The changes in gene expression observed in the present study were fairly small in magnitude. When expressed as normalized ratios, the genes showing decreased expression in schizophrenia were changed from 18% to 23%, while the absolute magnitude of the changes in genes showing increased expression was negligible. In other words, the distributions of values were such that a substantial change in z-scores, especially in the direction of increased expression in schizophrenia, was produced by a very small change in

Table 4

Genes in common on neuroarray and PSYN, GABA and GLUTAMATE classifications as studied by [Mirnics et al. \(2000\)](#)

Gene name	Unigene cluster	z-Ratio
<i>GABA system (GABA)</i>		
Phosphodiesterase 1C, calmodulin-dependent (70 kDa) (PDE1C)	Hs.48324	− 0.71
Calcium/calmodulin-dependent protein kinase II	Hs.81499	− 0.67
Glutamate decarboxylase 1 (67 kDa) ^a	Hs.75668	− 0.66
Glutamate decarboxylase 2	Hs.1668	− 0.37
Calmodulin	Hs.101688	− 0.33
CaM kinase II isoform	Hs.5171	− 0.29
GABA-A receptor, beta 1	Hs.89768	0.07
GABA-A receptor, gamma 2	Hs.7195	0.10
GABA-A receptor pi subunit	Hs.70725	0.40
Neurotransmitter transporter, betaine/GABA, member 12	Hs.82535	0.73
Calbindin 1	Hs.65425	1.28
<i>Glutamate system (GLUT)</i>		
Glutamate receptor, ionotropic, AMPA2 ^a	Hs.89582	− 1.64
Glutamate receptor 2 (HBGR2)	Hs.1687	− 0.41
Glutamate receptor, ionotropic, N-methyl-D-aspartate 1	Hs.105	− 0.26
Glutamate receptor, metabotropic 3	Hs.108060	− 0.21
Glutamate receptor (GLUR5)	Hs.22631	− 0.13
Glutamate receptor, metabotropic 3	Hs.3786	0.03
NMDA receptor	Hs.36451	0.16
Glutamate receptor, ionotropic, kainate 5	Hs.2389	0.57
Glutamate receptor 1 (AMPA1)	Hs.7117	0.78
<i>Presynaptic function (PSYN)</i>		
Clathrin, light polypeptide (Lcb)	Hs.73919	− 1.39
N-ethylmaleimide-sensitive factor ^a	Hs.108802	− 1.33
Gamma SNAP	Hs.60415	− 1.02
Clathrin light chain A	Hs.104143	− 0.78
ATPase, H ⁺ transporting, lysosomal ^a (vacuolar proton pump) 42 kDa	Hs.86905	− 0.68
Synaptogyrin 1b 1C ^a	Hs.59191	− 0.51
Clathrin-associated/assembly/adaptor protein, large, beta1	Hs.74626	− 0.41
Voltage-gated calcium channel beta subunit	Hs.118081	− 0.37
Dynein, cytoplasmic, intermediate polypeptide 1	Hs.65248	− 0.33
Synaptobrevin2 (SYB2), vamp2	Hs.11209	− 0.31
SytV	Hs.23179	− 0.21
Syntaxin3	Hs.82240	0.01
RAB3A, member RAS oncogene family	Hs.27744	0.15
Cytoplasmic dynein intermediate chain 1	Hs.100222	0.17

Table 4 (continued)

Gene name	Unigene cluster	z-Ratio
<i>Presynaptic function (PSYN)</i>		
Syntaxin 5A	Hs.75923	0.33
Alpha SNAP	Hs.75848	0.42
DYNAMIN-1	Hs.126	0.49
ESTs, highly similar to dynein light chain, cytosolic	Hs.42333	0.49
Cytoplasmic dynein light chain 1	Hs.5120	0.54
Syntaxin7	Hs.29363	0.60
Synaptopodin	Hs.117778	0.96

^a Genes identified as showing a decreased expression in schizophrenia by [Mirnics et al. \(2000\)](#).

expression. These data suggest that the pools were well matched, in that the differences in gene expression were not widely distributed. These data, however, suggest that there are few large changes in gene expression that are associated with schizophrenia, and especially, that schizophrenia is not associated with increased expression of many genes. On the other hand, increased gene expression in schizophrenia could occur for genes that are not included in the neuroarray. It is perhaps worth noting that greater than 95% of the human genome is not represented in the neuroarray. The possibility that schizophrenia involves very large changes in expression of a few genes, accompanied by concurrent or consequent small variations in a much greater number of genes cannot be ruled out.

The most consistently changed gene was HINT, which codes for a protein of unknown function initially identified on the basis of an interaction with protein kinase C, but which lacks protein kinase C inhibitory activity ([Brenner et al., 1999](#); [Gilmour et al., 1997](#)). Although little is known about the physiological function of the HINT protein, there are indications that HINT and related proteins are involved in apoptosis or cellular stress ([Brenner et al., 1999](#); [Gamble et al., 2000](#)). It is interesting, therefore, that several other genes identified as being either up-regulated by z-ratios or down-regulated in the present study are involved in apoptotic pathways. MAPK14, which is the p38 MAP kinase ([Mielke and Herdegen, 2000](#)), TNFRSF6, also known as FAS, which initiates apoptosis via interacting with the FAS ligand ([Becher et al., 1998](#)), and NAIP ([Liston et al., 1996](#)) are directly involved in apoptosis in the nervous system. UBE2N may also be involved in apoptosis via regulation of p53 stability by ubiquitin

(Tan et al., 2000). The NDUFA4 gene product is involved in ubiquinone metabolism, and consequently in oxidative stress and possibly apoptosis (Kelso et al., 2001). Furthermore, the HINT gene is located at chromosome 5q31.2, near a possible schizophrenia vulnerability locus (Baron, 2001; Straub et al., 1997).

Decreased AMPA receptor protein or mRNA expression has previously been reported in schizophrenia (Eastwood et al., 1995, 1996, 1997; Gao et al., 2000; Meador-Woodruff and Healy, 2000), although a few studies (Freed et al., 1993; Healy et al., 1998) found no change. Interestingly, clozapine, a potent atypical neuroleptic, up-regulates AMPA receptor density in rats (Spurney et al., 1999) and changes in AMPA receptors were suggested to mediate the antipsychotic effects of neuroleptic drugs (Freed, 1989). The decrease in AMPA receptor mRNA expression seen in the present study and prior datasets (Mirmics et al., 2000) is consistent with hypoglutamatergic neurotransmission in the PFC in schizophrenia.

In another recent microarray study, Mirmics et al. (2000) identified genes in PSYN, GABA, and GLUT categories as being decreased in schizophrenia. The present study also showed decreases in the average expression of AMPA2 and the GABA-A receptor delta subunit which fall within the general categories of GLUT and GABA genes, respectively (we do not believe that the GABA-A receptor delta subunit is included in the Incyte GEM arrays used by Mirmics et al., 2000). In addition, we were able to identify the same alterations reported earlier by Mirmics et al. (2000). A pattern of decreased expression of certain genes associated with presynaptic function, glutamatergic neurotransmission, and GABAergic neurotransmission was observed, which was highly consistent with the genes identified by Mirmics et al. (2000) as showing decreased expression in schizophrenia.

We did not, however, detect changes in the expression of myelination-related genes as recently reported by Hakak et al. (2001). Another recent microarray study by Mirmics et al. (2001) reported changes in the expression of regulator of G-protein signaling 4 (RGS4) in schizophrenia. RGS4 was not included in the neuroarray. RGS1, RGS5, RGS7, and RGS13 were included in the neuroarray. RGS5 and RGS7 showed a trend towards decreased expression in schizophrenia, while there was no difference in RGS1 or RGS13. Whether the changes presently identified are directly

related to schizophrenia etiology, or reflect neuroleptic treatment prior to death, or other factors such as cigarette smoking, cannot be determined presently. Future studies will examine gene expression differences in individual subjects using both microarrays and independent techniques to verify these suggested differences.

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