A modified method of differential display was employed to identify a novel gene (named PSZA11q14), the expression of which was reduced in brains from patients with schizophrenia. Decreased expression of PSZA11q14 was identified initially in Brodmann’s area (BA) 21 from a small group of patients with schizophrenia ($n = 4$) and normal controls ($n = 6$) and was confirmed subsequently using independent RT-PCR assay in BA 21, 22, and 9, and in hippocampus from a larger group of patients with schizophrenia ($n = 36$) and controls ($n = 35$). PSZA11q14 is located on chromosome 11q14, an area shown previously to co-segregate with schizophrenia and related disorders in several families. Decreased expression of PSZA11q14 in patients with schizophrenia and its location on 11q14 provide converging lines of evidence indicating that PSZA11q14 may be involved in at least some cases of schizophrenia. PSZA11q14 shows no significant homology with any known gene. It has no introns and produces two RNA transcripts of ~4.5 and ~7.0 kb. The largest open reading frame (ORF) in the PSZA11q14 transcripts may potentially encode for a short polypeptide of 71 amino acids. High frequency of rare codons, the short size of this ORF, and low homology with mouse sequences, however, indicate that PSZA11q14 may instead represent a novel member of a family of nonprotein-coding RNA genes that are not translated and that function at the RNA level. PSZA11q14 is located within the first intron of the LG-2 gene and transcribed in the opposite direction to DLG-2. These results suggest that PSZA11q14 may be considered a candidate gene for schizophrenia acting as an antisense regulator of DLG-2, which controls assembling functional N-methyl-D-aspartate (NMDA) receptors.

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1996; Sutherland et al., 1996; Tycowski et al., 1996; Wervick and Francke, 1997; Lee et al., 1999; Morelli et al., 2000). Some of these nonprotein-coding RNA genes have been implicated in human genetic disorders (Brockdorff et al., 1992; Leighton et al., 1995; Penny et al., 1996; Sutherland et al., 1996; Lee et al., 1999; Morelli et al., 2000). Currently, there is no algorithm to predict these unusual nonprotein-coding RNA genes from genomic sequences, and the majority of them remain to be discovered. It is possible that nonprotein-coding RNA genes are involved in schizophrenia, providing a rationale for using screening methods that are not biased against them. We have developed previously a variant of the differential display method based on random (not polyA-tail targeted) priming in PCR (Sokolov and Prockop, 1994). This method allows unbiased analysis of any RNA transcripts present in the cell, permitting identification of novel and unusual genes. In addition, like other variants of the differential display method and unlike microarray techniques currently in use, it can identify low abundance transcripts (Liang and Pardee, 1992, 1998) and may be a useful additional tool for studying molecular changes in schizophrenia.

Genetic studies have identified several chromosomal regions that co-segregate with schizophrenia (Levinson et al., 1998; Bailer et al., 2000; Gurling et al., 2001). One of the most significant and replicable findings has been cosegregation at 11q14. Max LOD score of 6.0 was reported for a balanced translocation t(1:11)(q42.1, q14.3) in a large Scottish family (St Clair et al., 1990; Millar et al., 2000). A LOD score of 3.4 for markers at 11q14 was reported in a large unrelated Canadian family (Maziade et al., 1995) and a LOD score of 3.2 was reported in a study of thirteen Icelandic and British families with schizophrenia (Gurling et al., 2001). In several small families, positive but less significant LOD scores have been reported (Smith, 1989; Holland and Gosden, 1990; Nanko et al., 1992; Craddock et al., 1994; Levinson et al., 1998). These genetic findings indicate strongly that a chromosomal region near 11q14 may contain a gene involved in some cases of schizophrenia. No linkage between schizophrenia and markers near 11q14 were found in several other families (Gill et al., 1993; Su et al., 1993; Wang et al., 1993; Hovatta et al., 1994; Karayiorgou et al., 1994; Kalsi et al., 1995; Mulkern et al., 1995; Bailer et al., 2000; Maziade et al., 2001), indicating that 11q14 may play a role only in a subset of schizophrenic cases.

The current study used a modified variant of the random priming differential display method (Sokolov and Prockop, 1994) to identify a novel gene located on 11q14, which is abnormally expressed in the brain of patients with schizophrenia. The gene (tentatively named PSZA11q14, a putative schizophrenia associated gene from 11q14) displays features consistent with those of nonprotein-coding RNA genes described above. We hypothesize that PSZA11q14 may be involved in some cases of schizophrenia.

### MATERIALS AND METHODS

**Patients**

Postmortem tissue derived from three cortical regions (middle and superior temporal gyrus and middle frontal gyrus corresponding to Brodmann’s areas [BA] 21, 22, and 9, respectively) and hippocampus of 35 normal controls and 36 individuals with schizophrenia (DSM-III-R/DSM-IV) were closely matched for gender (F:M, 13:22 [controls] vs. 13:23 [schizophrenics]) and hippocampus of 35 normal controls and 36 individuals with schizophrenia (DSM-III-R/DSM-IV) were closely matched for gender (F:M, 13:22 [controls] vs. 13:23 [schizophrenics]) and brain pH (mean ± SEM, 6.2 ± 0.1 [controls] vs. 6.3 ± 0.1 [schizophrenics]). Age was greater in persons with schizophrenia than in controls (55 ± 3 years; range, 16–96 years vs. 64 ± 3 years; range, 25–95 years, respectively; t = −2.12, df = 69, P = 0.037). Summary of demographic data for the diagnostic groups studied is shown in Table I. One patient had no record of treatment with neuroleptics. In other cases, neuroleptic-free intervals before death varied from less than 72 hr to more than 5 years. The postmortem interval delay (PMI) among controls (15.1 ± 1.8 hr; range, 3–42 hr) was shorter (t = −4.50, df = 70, P < 0.001) than that among patients with schizophrenia (40.2 ± 5.4 hr; range, 3.5–111.1 hr). Accordingly, to confirm decreased expression of PSZA11q14 we chose reverse transcription PCR (RT-PCR), a method known to be relatively insensitive to partial RNA degradation associated with prolonged PMI and other artifacts (Ma et al., 1994; Zamorano et al., 1996; Sokolov, 1998; Stanta and Bonin, 1998; Sokolov et al., 2000). In this method, the abundance of an mRNA of interest is estimated from amplification of a short fragment of the mRNA. Thus, even partially degraded mRNA

### Table I. Demographics for Controls and Groups of Patients With Schizophrenia*

| Tissue sample origin | Diagnostic group | Gender (F/M) | Age (years) | PMI (hr) | Brain pH | PSZA11q14 RNA levels in BA21 |
|----------------------|------------------|--------------|-------------|----------|----------|-----------------------------|
| Mount Sinai          | Controls (n = 10) | 4/6          | 77.4 ± 3.62 (55–96) | 5.3 ± 0.72 (3–10) | 6.1 ± 0.1 (5.5–6.7) | 1.54 ± 0.36 (0.51–4.26) |
|                      | Patients (n = 24) | 9/15         | 74.3 ± 2.38 (52–95) | 44.1 ± 7.72 (3.5–111.1) | 6.3 ± 0.1 (5.8–7.1) | 0.80 ± 0.14 (0.25–2.75)* |
| Stanley              | Controls (n = 15) | 6/9          | 48.1 ± 2.75 (29–68) | 23.7 ± 2.57 (8–42) | 6.3 ± 0.1 (5.8–6.6) | 2.29 ± 0.15 (1.71–4.14) |
|                      | Patients (n = 12) | 4/8          | 44.7 ± 3.90 (25–62) | 32.5 ± 4.08 (12–61) | 6.2 ± 0.1 (5.8–6.6) | 2.21 ± 0.20 (1.46–4.10) |
| Institute of Brain   | Controls (n = 10) | 3/7          | 43.1 ± 4.45 (16–38) | 12.2 ± 1.38 (5–19) | 6.3 ± 0.1 (6.1–6.5) | 1.85 ± 0.32 (0.52–3.66) |
| Total                | Controls (n = 35) | 13/22        | 55.0 ± 3.14 (16–96) | 15.1 ± 1.78 (3–42) | 6.2 ± 0.1 (5.5–6.7) | 1.95 ± 0.16 (0.51–4.26) |
|                      | Patients (n = 36) | 13/23        | 64.4 ± 3.10 (25–95) | 40.2 ± 5.36 (3.5–111.1) | 6.3 ± 0.1 (5.8–7.1) | 1.33 ± 0.17 (0.25–4.10) |

*Data are means ± SEM; minimum and maximum values are shown in parentheses. Data for PSZA11q14 levels in BA22, BA9, and hippocampus were available only for samples from the Mount Sinai Brain Bank and were shown in Figure 3.

†Data are for 20 persons with schizophrenia from whom tissue from BA21 was available.
molecules are accounted for in the measurements. Additionally, these determinations are normalized with measurements of a similar size fragment of an endogenously expressed housekeeping mRNA that is co-amplified in the same RT-PCR reaction. The procedure helps to account for possible differential levels of RNA degradation among the samples. As expected based on the principals described above and in greater detail in several published studies (Ma et al., 1994; Zamorano et al., 1996; Sokolov, 1998; Stanta and Bonin, 1998; Sokolov et al., 2000), no significant effect of PMI on PSZA11q14 RNA measurements was observed when using RT-PCR assay protocols described below (see Table II). In addition, analyses limited to cases with equivalent mean PMI and PMIs shorter than 24 hr (controls and persons with schizophrenia similar to those found elsewhere) revealed differences in PSZA11q14 expression between controls and persons with schizophrenia similar to those found between entire control and schizophrenic groups (controls vs. schizophrenics: BA21, 1.78 ± 0.19 vs. 1.10 ± 0.22; BA22, 1.54 ± 0.38 vs. 0.80 ± 0.10; BA9, 1.43 ± 0.28 vs. 0.95 ± 0.21; hippocampus, 1.84 ± 0.50 vs. 1.03 ± 0.51) reaching statistical significance in BA21 at P = 0.03 (two-tailed t-test). These data strongly indicate that differences between schizophrenics and controls reported here are unlikely to have been due to variations in PMI. There were no statistically significant correlations between brain pH and PSZA11q14 mRNA measurements using the RT-PCR method (see Table II for details). Brain tissue from 24 schizophrenics and 10 controls was obtained from the Department of Psychiatry Brain Bank at the Mount Sinai School of Medicine. Brain tissue from 12 schizophrenics and 15 controls was obtained from the Stanley Foundation, and tissue from 10 additional controls was obtained from the Institute of Brain (St. Petersburg, Russia). These cases have been described previously in detail (Hernandez and Sokolov, 2000; Torrey et al., 2000). Samples were not available for all cases in all regions. Genotyping was carried out for DNA from brain tissue of 12 normal controls (11 Caucasians and 1 African–American) and 13 patients with schizophrenia (11 Caucasians and 2 Asians) and for DNA from peripheral leukocytes from 34 normal unrelated Caucasians selected randomly. All specimens were provided to us without personal information that could be used to identify the donors.

**Isolation of Total RNA and DNA**

Total RNA and DNA were isolated from 200–500 mg of sample using TR1 Reagent (MRC, Cincinnati, OH). RNA was treated with DNase and reverse transcribed using SuperScript kit (Gibco BRL, Grand Island, NY) as described previously (Sokolov, 1998). Poly(A)+ mRNA was isolated from total RNA using two rounds of selection on columns of oligo(dT)–cellulose purchased from Clontech (Palo Alto, CA).

**Differential Display**

Differential display was carried out as described previously (Sokolov and Prockop, 1994) with minor modifications. Total RNA was reverse transcribed using a fully degenerated 6-mer oligonucleotide as primer and amplified by PCR. Thermocycling was at 94°C for 30 sec, 37°C for 2 min, and 72°C for 1 min on a 9600 (Perkin Elmer, Foster City, CA) temperature cycler. In total, 50 cycles were employed, followed by 10 min at 72°C. PCR products were electrophoresed in 5% polyacrylamide gel (PAAG) containing 2 M urea. DNA bands were visualized by ethidium bromide staining and cut from the gel. Gel pieces were washed thoroughly with water, frozen, and finely crushed. The DNA was extracted by freezing and thawing followed by centrifugation. The PCR products eluted from the gels were cloned using TA Cloning kit (Invitrogen, San Diego, CA). Each RNA sample was examined in two parallel PCR reactions using different amounts of cDNA (100 and 200 ng per 50 μL of PCR mixture). Ten different primer combinations, as described previously (Sokolov and Prockop, 1994), were used in ten experiments. One of these experiments (using primers BS71: 5′-GAGCTATGGCATG-3′ and BS74: 5′-AGCCTGTGT-CTGA-3′) identified a 562-base pair (bp) band with decreased expression in schizophrenics (Fig. 1).

**PSZA11q14 RNA Level**

Levels of PSZA11q14 RNA were measured as ratios to β-actin using an adaptation of the “switch-profile” RT-PCR

| Parameter | Value | PMI | PH |
|-----------|-------|-----|-----|
|           |       |     |     |
| BA21      |       |     |     |
| BA9       |       |     |     |
| BA22      |       |     |     |
| Hippocampus |     |     |     |
| Average   |       |     |     |
|           |       |     |     |
| PMI       |       |     |     |
| PH        |       |     |     |

*Data from the four brain regions for individual cases were averaged after normalization using mean values for the set of samples that were examined in all four brain regions.

Fig. 1. Section of a differential display gel containing a 562-bp PCR product of PSZA11q14 transcripts amplified from Brodmann’s area (BA) 21 of 4 patients with schizophrenia and 6 controls. Band intensity for PSZA11q14 is reduced significantly in 3 patients with schizophrenia. Note that due to the “smiling effect” the band for the 562-bp product is higher than in controls.
method described previously in detail (Sokolov, 1998; Hernandez and Sokolov, 2000). Despite different abundance of PSZA11q14 and β-actin mRNA, the use of the switch-profile method allows simultaneous amplification of the PSZA11q14 and β-actin mRNA in the same tube with similar kinetics, yields, and overlapping exponential phases (Fig. 2). Primers for PSZA11q14 mRNA were: C11-1F (5'-ATCCATCCTTGACTCAGGAATAG-3') and C10-1R (5'-CTTAAGTGTCACAGTCTCTTACGACC-3'). These primers were for the common sequence of the 4.5- and 7.0-kb transcripts of the PSZA11q14 transcript. Aliquots were examined first using the radioactive assay. Subsequently, these samples were reexamined together with samples from Mount Sinai using the non-radioactive assay. In each series of measurements, all samples were run simultaneously on the same 96-well plate using aliquots from the same master reaction mixture. Data from radioactive assay of samples from Mount Sinai were normalized to data from the non-radioactive assay using mean values for all samples run by both assays. Up to four independent measurements were carried out for each individual sample and data were averaged for the analysis.

3'- and 5'-RACE

3'-RACE and 5'-RACE were carried out using commercial kits (Gibco BRL) according to the manufacturer's specifications. Northern blotting was carried using filters containing 2 μg of poly(A) + RNA per lane (MTN blots I and II; Clontech). Dot-blot hybridization was carried out using the Human RNA Master Blot (Clontech) containing poly(A) + RNA from 50 human tissues.

Chromosomal Localization of the PSZA11q14 Gene

DNA from the PI clone containing PSZA11q14 was labeled with digoxigenin dUTP by nick translation and hybridized to normal metaphase chromosomes derived from peripheral blood lymphocytes. The initial experiment resulted in specific labeling of the long arm of a group C chromosome believed to be chromosome 11 based on size, morphology, and banding pattern. A second experiment was conducted in which a biotin-labeled probe specific for the centromere of chromosome 11 was co-hybridized with clone PSZA11q14. Measurements of ten specifically labeled chromosomes 11 demonstrated that PSZA11q14 was located at the position that is 44% the distance from the centromere to the telomere of chromosome arm 11q, an area corresponding to band 11q14. Of 80 metaphase cells analyzed, 76 exhibited specific labeling.

Genotyping of Polymorphisms

Genotyping of polymorphisms was carried out by amplification of a 1,196-bp fragment of the PSZA11q14 gene using primers C11-1F (5'-ATCCATCCTTGACTCAGGAATAGACC-3') and C10-1R (5'-CTTAAGTGTCACAGTCTCTTACGACC-3'). The conditions for PCR were: 25 cycles consisting of 94°C for 30 sec, 58°C for 30 sec, and 72°C for 60 sec. The products were separated by electrophoresis in 5% PAAG DNA, fragments were quantified by incorporated radioactivity (radioactive assay) or by SYBR Green (FMC, Rockland, ME) staining (non-radioactive assay) using PhosphorImager (Molecular Dynamics). Samples from Mount Sinai and the Institute of Brain were examined first using the radioactive assay. Subsequently, these samples were reexamined together with samples from Stanley Consortium using the non-radioactive assay. In each series of measurements, all samples were run simultaneously on the same 96-well plate using aliquots from the same master reaction mixture. Data from radioactive assay of samples from Mount Sinai were normalized to data from the non-radioactive assay using mean values for all samples run by both assays. Up to four independent measurements were carried out for each individual sample and data were averaged for the analysis.

Sequence Similarity

A sequence similarity search was carried out using BLASTN and PSI-BLAST. Gene features were analyzed using Promoter Prediction, TestCode, TSSG, and CodonPreference programs (Wisconsin Package) from GCG (Madison, WI).
Statistical Analysis

Differences between controls and schizophrenics were examined using two-tailed Student’s t-test. The effects of PMI, brain pH, age, and withdrawal from neuroleptic treatment were examined using Pearson’s Product-Moment correlation analysis. ANOVA with brain region, sample origin, and other demographics as factors or co-variates was not carried out because samples were not available in all regions in all subjects, because some demographics (i.e., neuroleptic withdrawal) were not applicable to all samples (i.e., controls), and because only control cases were present among samples from the Institute of Brain. Statistical analyses were carried out using SPSS 10.0 and GB-Stat statistical programs.

RESULTS

Identification of Abnormally Expressed mRNA in Schizophrenic Brains Using Differential Display

A modified method of non-radioactive differential display was employed to screen for genes expressed abnormally in schizophrenia using postmortem brain tissue derived from BA21 of 4 patients with schizophrenia and 6 controls. Ten different primer combinations, described previously (Sokolov and Prockop, 1994), were used in ten independent experiments (each with two different mRNA concentrations), which together displayed expression of approximately 600 mRNAs. One primer combination identified a 562-bp fragment of an mRNA with apparently decreased expression in schizophrenics compared to normal controls (Fig. 1). This RNA was designated PSZA11q14 (schizophrenia associated RNA from 11q14). Note that extension 11q14 was added to the name of this RNA after the encoding gene was localized to chromosome 11, region 11q14 (see below).

The 562-bp fragment was cloned and its sequence was used to design an independent specific RT-PCR assay for the PSZA11q14 RNA (Fig. 2). Using this assay, relative expression of PSZA11q14 RNA was measured in three cortical regions (BA 21, 22, and 9) and in hippocampus from a large group of patients with schizophrenia (n = 36) and from normal controls (n = 35). Consistent with results of differential display analysis (Fig. 1), PSZA11q14 RNA levels were lower in schizophrenics than in normal controls for all four brain regions studied (Fig. 3). Reaching statistical significance in BA21 (t = -2.97, df = 65, P = 0.008) and hippocampus (t = -2.30, df = 30, P = 0.029), with nearly significant trends in BA9 (t = -1.78, df = 39, P = 0.08) and BA22 (t = -1.57, df = 30, P = 0.127). Additional analysis in which data from the four brain regions in individual cases were averaged (after normalization using mean values for sets of samples that were examined in all four regions and mean value in BA21 as a reference) confirmed significant differences between patients with schizophrenia and controls (t = -2.97, df = 69, P = 0.004). When PSZA11q14 mRNA levels in BA21 of schizophrenic patients from different sources were examined separately (Table I), PSZA11q14 expression was decreased in schizophrenic patient samples from the Mount Sinai Brain Bank (0.80 ± 0.14 vs. 1.54 ± 0.36, t = -2.32, df = 28, P = 0.028) but not from the Stanley Consortium (2.21 ± 0.20 vs. 2.29 ± 0.15). Samples from BA9, BA22, and hippocampus for cases from the Stanley Consortium were not available for this study.

There were no significant associations between PSZA11q14 RNA and gender in controls (BA21: t = 0.52, df = 33, P = 0.61; BA9: t = 0.27, df = 18, P = 0.79; BA22: t = -1.07, df = 16, P = 0.30; hippocampus: t = -2.06, df = 14, P = 0.06; t-test) or persons with schizophrenia (BA21: t = -0.79, df = 30, P = 0.44; BA9: t = 0.82, df = 19, P = 0.42; BA22: t = -0.51, df = 12, P = 0.62; and hippocampus: t = 1.58, df = 14, P = 0.14; t-test). Similarly, no significant gender differences were found when data from the four brain regions were averaged for analysis (controls: t = -0.150, df = 33, P = 0.882; persons with schizophrenia: t = -0.166, df = 34, P = 0.869). No significant effect of age was found in controls in any of the brain regions examined separately (see Table III). A significant inverse correlation between PSZA11q14 RNA level and age was found when data from the four brain regions were averaged (r = -0.35, n = 35, P = 0.039). This finding in data averaged from the four brain regions should be interpreted with caution, however, because for some cases averaged data represented the mean of all four brain regions, whereas for some other cases data represented measurements in only a single brain region. In addition, putative age effect may vary in different regions. In persons with schizophrenia, PSZA11q14 RNA level correlated inversely and significantly with age in BA21 (r = -0.65, n = 32, P < 0.001), whereas a non-significant trend toward inverse correlation with age was observed in BA22, BA9, and hippocampus (see Table III). Mean age in the entire group of persons with schizophrenia was greater than in the entire group of controls (Table I; t = -2.12, df = 69, P = 0.037), which might
influence the results of group comparison. It is important therefore to note that a significantly decreased level of PSZA11q14 RNA was revealed in BA21 from persons with schizophrenia from the Mount Sinai Brain Bank (0.80 ± 0.14 vs. 1.54 ± 0.36, \( t = -2.32, d.f = 28, P = 0.028 \)), who were well matched in age with controls from the Mount Sinai Brain Bank (75.1 ± 2.7 vs. 77.4 ± 3.62, \( t = 0.50, d.f = 28, P = 0.621 \)). This finding from age-matched cases confirms decreased expression of PSZA11q14 schizophrenic patient samples from the Mount Sinai Brain Bank.

There was no significant correlation between PSZA11q14 mRNA and time without neuroleptic treatment before death (range of less than 72 hr to greater than 5 years; BA21: \( r = 0.16, n = 21, P = 0.62 \); BA9: \( r = 0.08, n = 21, P = 0.74 \), BA22: \( r = -0.20, n = 14, P = 0.49 \); and hippocampus: \( r = 0.38, n = 16, P = 0.14 \); average in four brain regions: \( r = 0.25, n = 25, P = 0.231 \)) indicating that decreased expression of PSZA11q14 in persons with schizophrenia was not associated with acute neuroleptic treatment.

**Chromosomal Location, PSZA11q14 Gene Structure, and Encoded Transcripts**

A genomic clone containing the PSZA11q14 gene was identified by PCR screening of a P1 library. In situ hybridization using this clone produced a strong signal on the long arm of chromosome 11, band 11q14 (Fig. 4A). No hybridization signal on other chromosomal regions was detected, indicating that PSZA11q14 has no duplications, pseudogenes, or otherwise highly homologous genes. A total of approximately 10 kb of PSZA11q14 and surrounding regions were sequenced (GenBank accession number AF525782; note that the PSZA11q14 gene was tentatively named SZ1 at the time of submission to GenBank). After chromosomal localization and sequencing of PSZA11q14 were completed, an anonymous genomic sequence reportedly derived from 11q14 and showing >99% of homology with PSZA11q14 was published (GenBank accession number AP001825) allowing us a more detailed localization of PSZA11q14 to 11q14 within the first intron of the DLG-2 gene using NCBI human genome databases (Fig. 4B).

Northern blotting using poly(A)+ RNA from various tissues revealed a major RNA band of ~4.5 kb, and an additional, minor band of ~7.0 kb (Fig. 5). The 3’-ends of the 4.5- and 7.0-kb transcripts were determined by 3’-RACE. Concordance of 3’-RACE derived cDNA sequences with the location of putative polyA signals in the genomic sequence together with detection of the 4.5- and 7.0-kb transcripts in Northern blotting of poly(A)+ RNA, which was enriched using two rounds of selection on columns of oligo(dT)-cellulose, indicated that the 4.5- and 7.0-kb transcripts are polyadenylated. The use of 5’-RACE produced 5’-RNA sequences identical to the genomic sequence of PSZA11q14. Thus, the 4.5- and 7.0-kb transcripts are not spliced and apparently result from alternative use of two different polyA signals (Fig. 6A). The existence of both the 4.5- and 7.0-kb PSZA11q14 transcripts was confirmed further by RT-PCR amplification (Fig. 6B). Extensive RT-PCR analysis using RNA from adult human brain (data not shown) provided no evidence for splicing of the PSZA11q14 transcripts.

Computer analysis using Promoter Prediction (Reese, 2001) and TSSG (Solovyev and Salamov, 1997) programs identified a putative PSZA11q14 promoter (Fig. 6A) with an “obligatory” TATA-box, CCAAT- and CG-rich sequences, as well as consensus sequences for multiple binding sites for several common regulatory factors (HNF-5, Gcn4, and TFIID), and a factor involved in neuronal apoptosis (MEF-2) (Okamoto et al., 2000). In addition, multiple binding sites for factors involved in cell proliferation and inflammation-related transcription (NF-κB, PEA3, and E1A-F) were predicted.

The two ORFs deduced to be present within the 4.5- and 7.0-kb RNA transcripts of the PSZA11q14 gene could potentially encode for two short oligopeptides that are 83 and 76 amino acids long (not shown). The putative start codons (ATG) in these two ORFs, however, are not surrounded by the Kozak consensus sequences. In addition, both ORFs carry internal ATG codons, indicating that the ORFs are not likely to be translated. The next longest ORF deduced to be present within the 4.5- and 7.0-kb transcripts of PSZA11q14 (Fig. 6A) encodes for a short polypeptide of 71 amino acids. The putative start codon (ATG) for initiator methionine of this ORF is surrounded by the Kozak consensus sequences (Kozak, 1987), and this ORF does not have internal ATG codons.

### Table III. Pearson’s Product-Moment Correlation Analysis of Age Effects on PSZA11q14 Transcript Levels

| Diagnostic group | Value | BA21 | BA9 | BA22 | Hippocampus | Average* |
|------------------|-------|------|-----|------|-------------|----------|
| Controls         |       |      |     |      |             |          |
| \( r \)          | −0.227| −0.377| −0.011| −0.296| −0.351      |          |
| \( P \)          | 0.189 | 0.102| 0.967| 0.265| 0.039       |          |
| \( n \)          | 35    | 20   | 18  | 16   | 35          |          |
| Patients         |       |      |     |      |             |          |
| \( r \)          | −0.652| −0.249| −0.408| −0.116| −0.704      |          |
| \( P \)          | <0.001| 0.276| 0.148| 0.670| <0.001      |          |
| \( n \)          | 32    | 21   | 14  | 16   | 36          |          |

*Data from the four brain regions for individual cases were averaged after normalization using mean values for the set of samples that were examined in all four brain regions.
These features indicate that this 71-amino acid long ORF may be translated. Analysis using the TestCode computer program revealed, however, that this ORF displayed an unusually high frequency of rare codons (TestCode value of 0.659) indicating that it was a nonprotein-coding sequence (Fickett, 1982). Furthermore, the nucleotide sequence of this ORF displayed only 48% homology with the corresponding mouse sequence, and had no bias toward enhanced variation at the third position of codons. Predicted protein homology for human and mouse ORFs was less than 30%. These features, along with the short ORF length, indicated strongly that the ORF is not translated, raising the possibility that PSZA11q14 belongs to a family of nonprotein-coding mRNA-like RNA genes (Storz, 2002). PSI-BLAST search revealed no significant similarities between the putative 71-amino acid polypeptide and any known or predicted protein. The use of a human PSZA11q14 probe in Northern hybridization detected no homologous mRNA in mice (data not shown). In addition, sequence analysis of PSZA11q14 revealed low homology (less than 50%) with the corresponding sequence in mouse genome, indicating that PSZA11q14 is poorly conserved among species.

Tissue-Specific Expression of the PSZA11q14 Gene

Northern blotting (Fig. 5) indicated that the 4.5- and 7.0-kb PSZA11q14 transcripts were of low abundance. RT-PCR experiments using co-amplification of PSZA11q14 and β-actin mRNAs indicated that SZ-1 transcripts were at least 10–50 times less abundant than β-actin mRNA (data not shown). Analysis using Northern blotting, dot-blot hybridization, and RT-PCR revealed low and variable expres-
Identification of Polymorphic Sites Within the PSZA11q14 Gene

Comparison sequences from different individuals, determined from sequencing of genomic clones containing PSZA11q14 and from sequencing of cDNA synthesized from commercial poly(A)+RNA from anonymous individuals, revealed a number of potential polymorphisms in PSZA11q14. Frequencies of two of these polymorphisms, C/T(3193), and CT/TG(3632/3), were examined using PCR followed by EcoRV and PstI restriction analysis (Table IV). The two polymorphisms displayed evidence for linkage disequilibrium (D = 0.989) that did not reach statistical significance (P = 0.09), probably due to the small sample size. Although statistical power of the sample was not enough to test accurately for association between polymorphisms and schizophrenia, the frequency of the rare allele T seemed decreased among schizophrenics (Table IV; differences were not statistically significant; \( \chi^2 = 2.097, df = 1, P = 0.147 \)).

Because many nonprotein-coding RNA genes are imprinted (Wevrick and Francke, 1997), the possibility of PSZA11q14 imprinting was examined in the temporal cortex from C/T(3193) and CT/TG(3632/3) heterozygous normal samples. No monoallelic expression was found in any of the cases, arguing against imprinting of PSZA11q14 (data not shown).

Discussion

We have identified and preliminarily characterized a novel gene (designated as PSZA11q14), the expression of which was decreased in the brain of patients with schizophrenia. The gene was discovered using the differential display method in postmortem tissue derived from one brain area (BA21) from a small group of persons with schizophrenia and normal controls. Decreased expression of this gene in schizophrenia was validated subsequently using an independent RT-PCR assay and tissue from three cortical areas (BA21, 22, and 9), and from hippocampus from a large cohort of patients with schizophrenia (n = 39) and from normal controls (n = 35). The decrease in PSZA11q14 RNA in schizophrenics was not associated with neuroleptic medication or variations in postmortem interval.

The PSZA11q14 gene has no introns and produces two RNA transcripts of ~4.5 and ~7.0 kb, apparently resulting from alternative 3'-end processing. PSZA11q14...
shows no significant homology with any known gene. The largest ORF in both the 4.5- and 7.0-kb transcripts is identical, and could potentially encode for a polypeptide of 71 amino acids with molecular weight of ~7.9 kDa. The potential start codon for initiator methionine of this ORF is in a good context, i.e., surrounded by the Kozak consensus sequences (Kozak, 1987). Whether or not the 71-amino acid polypeptide is expressed in vivo, however, remains to be established. Importantly, ORF encoding for the 71-amino acid polypeptide uses a high proportion of rare codons, which is unusual for translated sequences (Fickett, 1982). In addition, the sequence of this ORF is not conserved in the mouse genome, has no bias toward enhanced variation at the third position of codons and represents less than 5% of either of the 4.5- or 7.0-kb transcripts. These structural features suggest that

PSZA11q14 may belong to a family of RNA genes that are transcribed and may be polyadenylated, but they are not translated and they may function directly at RNA level (Brockdorff et al., 1992; Leighton et al., 1995; Hollander et al., 1996; Sutherland et al., 1996; Tycowski et al., 1996; Wevrick and Francke, 1997; Morelli et al., 2000). PSZA11q14 is a low abundance, ubiquitously expressed gene. In brain, the highest expression was found in hippocampus and amygdala. The lowest expression was in the occipital lobe, a brain region not associated commonly with schizophrenia.

In situ hybridization revealed that PSZA11q14 is located on the long arm of chromosome 11, band 11q14. This localization of PSZA11q14 on the 11q14 region is of particular importance. There is significant genetic evidence indicating that area of 11q14 may carry a gene involved in schizophrenia and related psychiatric disorders: A balanced translocation t(11:1)(q43,q14) with a breakpoint at 11q14 co-segregates with schizophrenia and related mental illnesses in a large Scottish family, resulting in a highly significant LOD score of 6.0 (St Clair et al., 1990; Millar et al., 2000). Three additional unrelated families with balanced 11q translocations t(6:11)(q14.2,q25) and t(9:11)(p22,q22.3) or partial trisomy of chromosome 11 co-segregating with non-specific psychosis or affective disorder have been reported, providing strong evidence that a putative gene associated with psychotic disorders in these families with 11q translocations is more likely to be located on chromosome 11 than on the other chromosomes involved in the translocations (chromosomes 1, 6, or 9) (Smith, 1989; Holland and Gosden, 1990; Craddock et al., 1994). Furthermore, a positive LOD score (3.4) for linkage with schizophrenia was reported for markers at 11q14–11q21 in a large French-Canadian pedigree (Maziade et al., 1995). A significant LOD score of 3.2 at 11q23.3–24 was reported in a recent study of 13 Icelandic and British families with schizophrenia (Gurling et al., 2001). Positive LOD score (1.5) for linkage with markers at q11 was reported in two Japanese pedigrees (Nankó et al., 1992). A genome scan of schizophrenia using multiple unrelated families revealed linkage with markers near 11q14 with marginal statistical significance (P < 0.05) (Levinson et al., 1998). Additionally, cosegregation of schizophrenia with tyrosinase-negative albinism caused by the TYR gene located at 11q14/21 (Fig. 4B) has been reported (Clarke and Buckley, 1989). Thus, a significant body of genetic evidence indicates that a region at or near 11q14 carries one or more genes that may be involved in schizophrenia. Localization of PSZA11q14 to 11q14 may therefore provide additional support for a possible role of the PSZA11q14 gene in schizophrenia. It should be mentioned, however, that genetic studies referenced above do not provide exact location of the gene or genes associated with schizophrenia. Instead, they indicate a large chromosomal region near 11q14 that harbors many genes.

Studies of several other families showed no linkage between schizophrenia and markers near 11q14 (Gill et al., 1993; Su et al., 1993; Wang et al., 1993; Hovatta et al., 1993; Leighton et al., 1995; Hollander et al., 1996; Sutherland et al., 1996; Tycowski et al., 1996; Wevrick and Francke, 1997; Morelli et al., 2000).
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was not decreased in patients from the Stanley Consortium, indicating that 11q14 may play a role in only a subset of schizophrenic cases. This is consistent with our finding that the expression of PSZA11q14 was decreased in the group of patients from the Mount Sinai Brain Bank but was not decreased in patients from the Stanley Consortium. Notably, these patient groups display significant clinical differences as well as differences in age. The Mount Sinai cases were severely affected elderly chronic patients with poor treatment response who had been hospitalized for many years. The cases from the Stanley Consortium were younger outpatients who lived with their families, in supported housing, or in group homes, and some were regularly employed. It may be speculated therefore that decreased expression of PSZA11q14 may be present only in some patients with a severe form of schizophrenia or it develops with aging of patients with schizophrenia.

As discussed above, features of PSZA11q14 indicate that it may belong to a family of nonprotein-coding, mRNA-like RNA genes (Brockdorff et al., 1992; Leighton et al., 1995; Hollander et al., 1996; Sutherland et al., 1996; Tycowski et al., 1996; Wevrick and Francke, 1997; Morelli et al., 2000). There is growing evidence that some of these nonprotein-coding RNA genes play significant roles in establishment or maintenance of a chromatin configuration necessary for appropriate expression of nearby genes and essential for epigenetic regulation of the genome (Brockdorff et al., 1992; Leighton et al., 1995; Lee et al., 1999). Abnormal function of these nonprotein-coding RNA genes may lead to abnormal expression of nearby genes and cause some human genetic disorders (Brockdorff et al., 1992; Leighton et al., 1995; Penny et al., 1996; Sutherland et al., 1996; Lee et al., 1999; Morelli et al., 2000). In fact, some of these genes were identified only because they cause human disorders; from genomic sequences, they likely would not be predicted to be functional because they do not encode for proteins. The effect of regulation by nonprotein-coding RNA genes may extend to very large chromosomal segments. For example, Xist and Tsix regulate complete silencing of the X chromosome (Penny et al., 1996; Lee et al., 1999).

PSZA11q14 is located within the first intron of the DLG-2 gene and is transcribed in the opposite orientation to DLG-2. It is possible therefore that PSZA11q14 acts as an antisense regulator of the DLG-2 gene. DLG-2, known also as chapsyn-110 or PSD-95, produces a protein located in postsynaptic densities where it directs clustering N-methyl-D-aspartate (NMDA) receptor subunits with ion channels to form functional NMDA receptors (Kim et al., 1996). NMDA receptors play important roles in many brain functions that are altered in schizophrenia. We hypothesize that abnormal expression of PSZA11q14 may cause abnormal expression of DLG-2, which may lead to abnormal structure of postsynaptic NMDA receptor-related densities and thus cause altered brain functions in schizophrenia. Interestingly, previous studies have reported abnormal mRNA expression of DLG-2 (Dracheva et al., 2001), NMDA receptor subunits (Sokolov, 1998; Ibrahim et al., 2000; Dracheva et al., 2001), and multiple synaptic proteins (Tcherepanov and Sokolov, 1997; Sokolov et al., 2000) in schizophrenic brains obtained from the Mount Sinai Brain Bank. The decreased expression of PSZA11q14 and the increased expression of DLG-2 in this group of persons with schizophrenia are consistent with the hypothesis that PSZA11q14 may be an antisense regulator of DLG-2.

In summary, this study identified a novel gene, which is expressed abnormally in the brain of some patients with schizophrenia, and is located in a chromosomal region 11q14 tightly linked with schizophrenia and related psychiatric disorders in several families. We suggest that this gene may be considered a candidate gene for schizophrenia. The function of this gene remains to be elucidated, and it is possible that PSZA11q14 serves as an antisense regulator of the DLG-2/PSD-95 gene. Several polymorphisms identified in PSZA11q14 will enable a more detailed analysis of genetic association between schizophrenia and PSZA11q14.

The findings here also show that differential display may be a useful alternative to microarray methods for screening for disease-associated genes. One advantage of differential display is that this method can identify low abundance transcripts (Liang and Pardee, 1992, 1998), which are very difficult to measure reliably using microarrays. Furthermore, differential display is not limited to known genes or ESTs, and may detect novel and unusual genes such as PSZA11q14.

Identification of PSZA11q14 also indicates that there might be a greater number of genes than envisioned previously that do not encode proteins, but which may play significant roles in and may be associated with human diseases. Because these genes are difficult to predict based...
on genomic sequence analysis (Eddy, 2002), they have only recently begun to attract significant attention.

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