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Expression of *ZNF804A* in Human Brain and Alterations in Schizophrenia, Bipolar Disorder, and Major Depressive Disorder: A Novel Transcript Fetally Regulated by the Psychosis Risk Variant rs1344706

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Abstract

Importance—The single-nucleotide polymorphism rs1344706 in the zinc finger protein 804A gene (*ZNF804A*) shows genome-wide association with schizophrenia and bipolar disorder. Little is known regarding the expression of *ZNF804A* and the functionality of rs1344706.

Objectives—To characterize *ZNF804A* expression in human brain and to investigate how it changes across the life span and how it is affected by rs1344706, schizophrenia, bipolar disorder, and major depressive disorder.

Design, Setting, and Participants—Molecular and immunochemical methods were used to study *ZNF804A* messenger RNA (mRNA) and ZNF804A protein, respectively. *ZNF804A* transcripts were investigated using next-generation sequencing and polymerase chain reaction–based methods, and ZNF804A protein was investigated using Western blots and immunohistochemistry. Samples of dorsolateral prefrontal cortex and inferior parietal lobe tissue were interrogated from 697 participants between 14 weeks' gestational age and age 85 years, including patients with schizophrenia, bipolar disorder, or major depressive disorder.

Main Outcomes and Measures—Quantitative measurements of *ZNF804A* mRNA and immunoreactivity, and the effect of diagnosis and rs1344706 genotype.

Results—*ZNF804A* was expressed across the life span, with highest expression prenatally. An abundant and developmentally regulated truncated *ZNF804A* transcript was identified, missing exons 1 and 2 ($ZNF804A^{E3E4}$) and predicted to encode a protein lacking the zinc finger domain. rs1344706 influenced expression of $ZNF804A^{E3E4}$ mRNA in fetal brain ($P = .02$). In contrast, full-length *ZNF804A* showed no association with genotype ($P > .05$). $ZNF804A^{E3E4}$ mRNA expression was decreased in patients with schizophrenia ($P = .006$) and increased in those with major depressive disorder ($P < .001$), and there was a genotype-by-diagnosis interaction in bipolar disorder ($P = .002$). ZNF804A immunoreactivity was detected in fetal and adult human cerebral cortex. It was localized primarily to pyramidal neurons, with cytoplasmic as well as dendritic and nuclear staining. No differences in ZNF804A-immunoreactive neurons were seen in schizophrenia or related to rs1344706 ($P > .05$).

Conclusions and Relevance—rs1344706 influences the expression of $ZNF804A^{E3E4}$, a novel splice variant. The effect is limited to fetal brain and to this isoform. It may be part of the mechanism by which allelic variation in *ZNF804A* affects risk of psychosis. *ZNF804A* is translated in human brain, where its functions may extend beyond its predicted role as a transcription factor.

The single-nucleotide polymorphism (SNP) rs1344706 within the zinc finger protein 804A gene (*ZNF804A*; GenBank NM_194250.1) (2q32.1) was the first to show unequivocal genome-wide association with schizophrenia and with a broader psychosis phenotype including bipolar disorder.1 These findings have been replicated2–4 and confirmed in meta-

analyses,5,6 and they beg the question of what the roles of *ZNF804A* and rs1344706 are in health and disease.

The gene's nomenclature reflects an amino-terminal sequence domain with homology to other members of the Cys2His2 family of zinc finger-binding transcription factors.7,8 However, empirical evidence for this role is lacking, other than preliminary data suggesting that *ZNF804A* affects the expression of some genes.9–11 Regarding the influence of rs1344706, the SNP (T → G, where T is both the major allele and the risk allele) resides in the second intron. As such, it is a noncoding polymorphism of unknown functionality, although some studies have reported imaging or behavioral correlates of the allele12–23 including differences in neural activity,12,14,20,22 white matter,18,19 and cognition.16,17 In molecular terms, the underlying mechanism likely involves a differential effect of the 2 alleles on gene regulation and transcript expression. The available data show no clear influence of rs1344706 on *ZNF804A* messenger RNA (mRNA) in adult brain,2,5,24 but an effect in the second trimester in utero has been reported.25

Despite these findings, many questions remain. First, for several psychosis risk genes, there is evidence that the risk SNPs affect specific (and previously unidentified) transcript isoforms via alternative splicing or promoter use.26 To our knowledge, this possibility has not been investigated for *ZNF804A*. Second, it is not clear whether *ZNF804A* expression is altered in schizophrenia or other psychiatric disorders.2,27 Third, whether *ZNF804A* is translated in human brain has yet to be shown. These issues are critical for understanding the pathophysiological significance of the genetic association of *ZNF804A* with psychosis. Herein, we report identification of a novel *ZNF804A* isoform in human dorsolateral prefrontal cortex (DLPFC) and map its expression across the life span. Notably, this isoform is selectively affected by rs1344706 in fetal brain, and it is also affected by diagnosis and diagnosis-bygenotype interactions. In contrast, the canonical *ZNF804A* transcript exhibits no genotype effects and only minor diagnostic differences. We also show that ZNF804A immunoreactivity is present in the human cerebral cortex, localized primarily to pyramidal neurons, and with no changes observed in schizophrenia.

Methods

Human Tissue

We studied *ZNF804A* transcripts in DLPFC from the National Institute of Mental Health (NIMH)/Lieber Institute for Brain Development series, including a lifetime cohort of nonpsychiatric controls from second trimester to 85 years old and patients with schizophrenia, bipolar disorder, and major depressive disorder (Table; see the article by Lipska et al28 for additional methods regarding demographic, clinical, and pathological descriptions). A subset of these postmortem fetal, infant, child, and adolescent brain tissue samples was provided by the National Institute of Child Health and Human Development Brain and Tissue Bank for Developmental Disorders. Another subset of the adult samples was donated to this study from the Stanley Brain Collection of the Stanley Medical Research Institute (see the article by Torrey et al29 for additional details on this collection). These samples were curated, processed, and dissected with methods identical to those used for the samples collected by the NIMH/Lieber Institute for Brain Development.

Gray matter was dissected out, snap frozen, and stored at −80°C prior to RNA extraction, which was carried out using standard methods. The DLPFC tissue from this series was also used for protein extraction and Western blotting. The second brain series, used for immunohistochemistry, came from the Stanley Medical Research Institute, who provided free-floating sections of inferior parietal lobe from men with schizophrenia and male control participants (eTable 1 in the Supplement).30 For this series, after brief formalin fixation of the cerebral hemisphere, 3- to 5-cm coronal slabs were cut, formalin fixed for 3 to 15 weeks, and stored in buffered 30% sucrose. All blocks containing inferior parietal lobe were serially cryostat sectioned at 60 μm. Participants from both series were genotyped for rs1344706 using TaqMan assay C_2834835_10 (Life Technologies). Immunohistochemistry was also carried out on slide-mounted sections cut from formalin-fixed, wax-embedded blocks of frontal, temporal, or visual cortex from 5 additional adults and of frontal cortex from 2 neonates. The NIMH/Lieber Institute for Brain Development brains were collected with audiotaped oral informed consent from legal next of kin under National Institutes of Health institutional review board protocol 90-M-1042. Studies in Oxford, United Kingdom, were approved by Oxfordshire National Health Service Research Ethics Committee B (#O02.040).

RNA Sequencing and 5′ Rapid Amplification of Complementary DNA Ends

We used RNA sequencing (RNAseq) to identify potentially new expressed transcripts of *ZNF804A* and to guide the construction of relevant primers for 5′ rapid amplification of complementary DNA (cDNA) ends (5′ RACE). We performed RNAseq on Clontech pooled poly(A)$^+$ mRNA from fetal and adult human brain to identify potential 5′ cDNA ends. After fragmentation, reverse transcriptase and random primers copied the cleaved fragments into first-strand cDNA. Second-strand cDNA was synthesized using T4 DNA polymerase, T4 polynucleotide kinase, and Klenow DNA polymerase, adding a single adenine base using a 3′ to 5′ exo$^-$ Klenow fragment, and ligating the paired-end adapters using T4 DNA ligase. An index (up to 12 nucleotides) was inserted into Illumina adapters so multiple samples could be sequenced in 1 lane of an 8-lane flow cell. Products were purified and enriched with polymerase chain reaction (PCR) to create the final cDNA library for highthroughput sequencing using the HiSeq 2000 system (Illumina). Results were mapped to GRCh37/hg19 using the TopHat2 splice-aware alignment software.31,32

We used the RNAseq read alignment data to design primer pairs to amplify *ZNF804A* transcripts. We first performed 5′ RACE in the commercial fetal and adult brain poly(A)$^+$ mRNA samples to validate the RNAseq results, with *ZNF804A*-specific antisense primers binding at exon 4, using SMART RACE cDNA Amplification and Advantage 2 PCR kits (Clontech). The poly(A)$^+$ mRNAs were reverse transcribed according to the manufacturer's protocol. The PCR amplification profile was as follows: 94°C for 2 minutes; 30 cycles of 94°C for 30 seconds, 70°C for 30 seconds, and 72°C for 2 minutes; and 72°C for 10 minutes after the last cycle. When necessary, nested PCR was done using gene-specific primers and a nested universal primer A. Having confirmed the 5′ cDNA ends, we used end-to-end PCR to validate the transcripts in the 2 samples by Advantage 2 PCR kits (Clontech) following the manufacturer's recommendations. All products were cloned into pCR4-TOPO (Invitrogen) and sequenced.

After confirming the presence of a truncated transcript of *ZNF804A* in the commercial fetal and adult brain poly(A)$^+$ mRNA samples, we performed end-to-end PCR in the DLPFC series (Table) to establish the presence of the truncated transcript in an independent sample and in a specific brain region.

Quantitative Reverse Transcription–PCR

Based on the results of the RNAseq, 5′ RACE, and end-to-end PCR, we designed primers to selectively detect the canonical *ZNF804A* transcript (Applied Biosystems; forward: TCTCAGCAAGAACGGGAACAA; reverse: CCAGAGCTTTTGCTATGGTATTTTC; probe: ACTCTGGACTATGCTGAGAA) and the newly identified transcript (Applied Biosystems; forward: CAAGCCAAAATGCGAGAAAATATT; reverse: CCTTGTCGAGAGGTAAACACAACA; probe: TTGTTAGAAGTGGATTGTCATGA), using Primer Express software (Applied Biosystems), for quantitative analyses by quantitative reverse transcription–PCR carried out using the TaqMan Gene Expression Assay on an ABI Prism 7900 system (Applied Biosystems) by the standard curve method. Samples were quantified in triplicate, with an initial denaturing step of 10 minutes at 95°C followed by 40 cycles of 15 seconds at 95°C and 60 seconds at 60°C. Expression was normalized to the geometric mean of 3 housekeeping genes (β-actin: assay Hs99999903_m1; β2-microglobulin: assay Hs99999907_m1; and β-glucuronidase: assay Hs99999908_m1). These analyses were conducted in the DLPFC samples (Table).

Western Blotting

Methods, antibodies, and experimental controls are detailed in the eAppendix in the Supplement. Briefly, protein extraction and Western blotting were carried out using standard techniques. We included not only protein extracts from adult and fetal human brain but also protein extracts from transfected and nontransfected HEK293 cells9 and competent bacteria expressing recombinant *ZNF804A*. The main anti-ZNF804A antibody used was a polyclonal antibody mapping to an internal epitope of human ZNF804A (D-14; Santa Cruz Biotechnology).

Immunohistochemistry

Immunohistochemistry was carried out as described in the eAppendix in the Supplement, using the avidin-biotin complex–diaminobenzidine method. Given the findings (see Results), we investigated whether ZNF804A immunoreactivity was affected by schizophrenia or rs1344706 using the sections of inferior parietal lobe (eTable 1 in the Supplement). We focused our analyses on lamina III pyramidal neurons, measuring their density, size, and staining intensity (eAppendix in the Supplement).

Statistical Analysis

Quantitative reverse transcription–PCR analyses were conducted in R version 3.0.1 software (R Foundation) using linear modeling, treating $\log_2$-transformed expression levels of each transcript as the outcome. Statistical models investigating age-related expression patterns in control participants covaried for RNA integrity number (RIN), sex, postmortem interval (PMI), and race. Subsequent models exploring the influence of these biological variables on

the expression of *ZNF804A* were adjusted for age as a B-spline with knots at 1, 10, 20, and 50 with an offset at birth to capture nonlinear patterns in expression. Statistical models investigating diagnosis-related expression patterns in adults covaried for sex, race, pH, PMI, and RIN; diagnosis-related expression fold changes were relative to the control group. Statistical models investigating genotype-by-diagnosis interactions included main-effect regression terms for genotype and diagnosis as well as an interaction term between the 2 variables to capture genotype effects within each diagnosis group. The statistical models for the genotype effect in fetal samples adjusted only for RIN and race, as the PMI of these samples was short (mean, 2.6 hours) and many samples were missing information on pH. All reported *P* values were calculated from *t* statistics computed from the log fold change and its standard error from each multiple regression model; therefore, they represent covariateadjusted *P* values.

For the immunohistochemical study, group comparisons were by unpaired *t* tests, and the influence of confounding variables was examined using Pearson correlations. Because of the small sample size (eTable 1 in the Supplement), G carriers were compared with TT homozygotes for statistical analysis.

Results

Identification of a Novel Truncated Transcript of *ZNF804A*

Using RNAseq, 5′ RACE, end-to-end PCR, and quantitative reverse transcription–PCR, we identified a novel human transcript of *ZNF804A* in fetal and adult whole-brain RNA and in DLPFC. It skips the first 2 exons and has a new 5′ untranslated region in intron 2 (approximately 20 kilobases downstream of rs1344706) (eFigure 1 in the Supplement). This isoform, denoted $ZNF804A^{E3E4}$, remains in frame and is predicted to encode an approximately 123-kDa protein that lacks the N-terminal 135 amino acids of *ZNF804A*, including the zinc finger domain located at residues 57 to 81. Given these findings, our quantitative analyses comprised separate assays for the 2 transcripts.

Effects of Age, Sex, and Other Factors on *ZNF804A* Transcripts Across the Life Span

Both *ZNF804A* transcripts were detected across the entire age range (Figure 1), being preferentially expressed fetally relative to postnatal life (*ZNF804A*: 1.98-fold higher expression, $P < .001$; $ZNF804A^{E3E4}$: 2.63-fold higher expression, $P < .001$). Overall, $ZNF804A^{E3E4}$ is more abundant than full-length *ZNF804A* mRNA (3.79-fold higher expression, $P < .001$). Their lifetime expression trajectories were similar—both transcripts decreased by similar amounts during infancy and childhood (11% decrease per year on average; full length: $P < .001$; truncated: $P = .01$) but remained constant across adulthood.

There was no main effect of sex on either transcript across the life span, but *ZNF804A* mRNA was more highly expressed in men compared with women (1.20-fold higher expression, $P = .02$). There were significant effects of both RIN and PMI on the full-length transcript (RIN: $P < .001$; PMI: $P = .03$), but only RIN was associated with levels of the truncated transcript ($P < .001$). There were differences in $ZNF804A^{E3E4}$ expression levels between the small set of Hispanic participants (n = 7; 1.90-fold lower expression, $P = .02$)

and Asian participants (n = 6; 1.85-fold higher expression, $P = .04$) compared with white participants; there were no differences between African American and white participants or any racial differences in the expression of the full-length transcript.

Both *ZNF804A* Transcripts Are Associated With Diagnosis

We examined whether the 2 transcripts are differentially expressed in patients with schizophrenia, bipolar disorder, and major depressive disorder in DLPFC. As shown in Figure 2, $ZNF804A^{E3E4}$ mRNA was increased in major depressive disorder (1.45-fold higher expression, $P < .001$) and decreased in schizophrenia (1.22-fold lower expression, $P = .006$). In contrast, full-length *ZNF804A* mRNA was unaltered in schizophrenia and major depressive disorder and reduced in bipolar disorder (1.17-fold lower expression, $P = .01$). The $ZNF804A^{E3E4}$ to *ZNF804A* mRNA ratio was associated with all 3 disorders: it was elevated in bipolar disorder ($P = .002$) and major depressive disorder ($P < .001$) and decreased in schizophrenia ($P = .001$). There were no significant diagnosis-by-sex interactions affecting either transcript.

Expression of *ZNF804A* mRNA did not correlate with antipsychotic or antidepressant exposure overall or within each diagnostic group (all $P > .10$), nor were these medications significant in multivariate regression models. $ZNF804A^{E3E4}$ mRNA was associated with antidepressant use across all participants (1.32-fold increase, $P = .001$) but not within any diagnostic group. Medication history did not bias the observed fold changes of expression between diagnostic groups, but the fact that only 413 participants (279 of 371 with a psychiatric diagnosis [75.2%]) had a recorded medication history reduced statistical significance (not shown).

Psychosis Risk SNP rs1344706 Affects Fetal $ZNF804A^{E3E4}$ mRNA Expression

The SNP rs1344706 did not affect expression of either transcript when all participants were analyzed together. However, in a planned analysis restricted to the fetal brains given the finding by Hill and Bray,25 we found a significant effect of rs1344706 on $ZNF804A^{E3E4}$ mRNA ($F = 6.07$; $P = .02$) (Figure 3), with participants homozygous for the risk T allele (n = 33) showing lower expression than G carriers (n = 9) ($P = .03$). In contrast, full-length *ZNF804A* mRNA showed no allelic effects in fetal or adult samples.

In post hoc comparisons, we also identified 1 genotype-by-diagnosis interaction: in the bipolar disorder group, TT homozygotes had lower expression of $ZNF804A^{E3E4}$ mRNA ($P = .002$) (eFigure 2 in the Supplement).

Detection and Distribution of ZNF804A Immunoreactivity

Western blots showed that ZNF804A immunoreactivity is present in adult and fetal brain (Figure 4A and eFigure 3 in the Supplement). A single band was identified at approximately 140 kDa, similar to that observed in *ZNF804A*-transfected cells and bacteria9 and corresponding to the predicted molecular weight of 137 kDa. Signal was abolished by the blocking peptide, and the P-13 mouse antibody produced the same banding pattern (eFigure 4 in the Supplement).

Immunohistochemistry showed staining for ZNF804A in human cerebral cortex sections (Figure 4B-F), especially in lamina III and concentrated over pyramidal neurons. Immunoreactivity was strong in the cytoplasm and proximal dendrites, often with a prominent granular appearance (Figure 4D-F). Some staining of nuclei was also observed. Other cell populations did not show consistent immunolabeling. Findings were similar in all cortical regions examined and were comparable between free-floating and slide-mounted sections. The pyramidal cell immunoreactivity was abolished by preincubation with the blocking peptide (eFigure 5 in the Supplement).

There were no differences in the density, size, or staining intensity of ZNF804A-immunoreactive neurons between patients with schizophrenia and controls or between rs1344706 genotypes (eTable 2 in the Supplement). Although staining intensity correlated inversely with storage time, the latter did not differ between diagnostic or genotype groups, and the group comparisons remained negative if staining intensity was included as a covariate.

Discussion

The association between rs1344706, located in an intron of *ZNF804A*, and psychosis is statistically robust but biologically unexplained, reflecting the lack of understanding of the roles of *ZNF804A* and the unknown functionality of the SNP. Herein, we report findings that advance both these questions. We identify a novel mRNA isoform, $ZNF804A^{E3E4}$, in human brain that appears selectively modulated by rs1344706 during fetal life. In contrast, full-length *ZNF804A* mRNA shows no genotype effects. In consequence, we propose that the $ZNF804A^{E3E4}$ isoform mediates, at least partly, the association of rs1344706 with psychosis. The relative expression of $ZNF804A^{E3E4}$ mRNA compared with the full-length transcript was decreased in schizophrenia and increased in major depressive disorder and bipolar disorder. We also provide the first evidence, to our knowledge, that *ZNF804A* is translated in human brain and is localized primarily to pyramidal neurons.

Prior to this study, only 1 *ZNF804A* transcript had been reported in human brain, although a rare isoform with a putative extra exon was identified in lymphoblasts of some individuals.33 Using RNAseq, a powerful technology for identifying novel splice variants,34,35 and confirming by 5′ RACE and end-to end PCR, we discovered the truncated isoform $ZNF804A^{E3E4}$, which lacks exons 1 and 2 and has a 5′ extension of exon 3. This transcript was abundant and remains in frame, suggesting that it represents an alternative proteincoding transcript of potential functional significance. Both mRNAs were more highly expressed in fetal brain but continue to be expressed throughout life, relative to endogenous control gene levels.

As risk genes for psychiatric disorders are discovered, revealing the mechanism for association becomes important. *ZNF804A* is typical of many such associations because the risk SNP is noncoding, and without a coding variant in linkage disequilibrium any functionality is likely mediated via an effect on gene expression. A recent study provided initial evidence for this in fetal but not adult brain.25 Our data confirm this observation and, critically, show that the effect of rs1344706 is on $ZNF804A^{E3E4}$ mRNA rather than on full-

length *ZNF804A*. The primers used in the earlier study amplify both variants, so the present data are both a replication and a clarification. Our finding that a psychosis risk SNP selectively affects expression of 1 transcript variant of a gene adds to an increasing list of such findings36–42 and supports the proposal that this may be a common feature, particularly for fetally enriched isoforms.26 It is also relevant that splice variants are being recognized as showing functional and therapeutically relevant differences from the canonical isoform,43–45 including examples from other schizophrenia-associated genes,39,46 and being mediated by various molecular mechanisms.44,47,48 As well as having an effect in fetal brain, rs1344706 also influenced expression within a diagnostic group, namely $ZNF804A^{E3E4}$ mRNA in bipolar disorder. The latter was not a planned analysis and hence the finding is preliminary, but it is notable that, as in fetal brains, the risk allele is associated with lower expression. The mechanism is unclear; presumably, rs1344706 interacts with other bipolar disorder–specific factors (whether genetic, epigenetic, or epiphenomenal) to modulate *ZNF804A* expression.

In addition to the influences of genotype, main effects of diagnosis were found. These again primarily affected $ZNF804A^{E3E4}$ mRNA rather than the full-length transcript. The diagnostic alterations do not appear to be linked directly to the genetic predisposition: the transcripts change in opposite directions in the 2 disorders with which *ZNF804A* is associated (decreased in schizophrenia, increased in bipolar disorder), and expression is also affected in major depressive disorder, for which there is no significant evidence of association with *ZNF804A*. The altered expression in the 3 disorders may in part result from antidepressant use (see Results), but this does not substantially explain the findings. We also did not see relationships with other disease-related variables (eg, duration, age at onset). Thus, the explanations for differential expression of $ZNF804A^{E3E4}$ mRNA in these disorders and the reduction of *ZNF804A* mRNA in bipolar disorder remain unclear and warrant additional research.

The presence of ZNF804A immunoreactivity indicates that the gene is not only transcribed but also translated in human brain. ZNF804A immunoreactivity was not concentrated in the nucleus as expected for a classic transcription factor and as reported in rodent neural progenitors.9 Its abundance in the cytoplasm suggests additional roles for *ZNF804A* in human brain; it also raises the possibility of cross-reactivity of the antibody to another protein, although this is unlikely given the experimental controls used. Neither rs1344706 nor schizophrenia affected our measures of ZNF804A immunoreactivity, so the penetrance of genotype and diagnostic effects through to ZNF804A protein remains to be determined. In any event, understanding the pathophysiological significance of *ZNF804A* is hindered by uncertainty about the translation and function of $ZNF804A^{E3E4}$. The variant is predicted to encode a 123-kDa protein; although no separate band of this size was seen on Western blots, it could be subsumed within the approximately 137-kDa band corresponding to full-length *ZNF804A* if $ZNF804A^{E3E4}$ undergoes posttranslational modifications that increase its apparent molecular weight. If it is translated, $ZNF804A^{E3E4}$ may well contribute to the non–transcription factor–related functions postulated here (because it lacks a zinc finger domain), but it does not contain strong sequence motifs or homologies that might give positive clues in this regard.

Conclusions

We describe a novel, truncated transcript of *ZNF804A*, $ZNF804A^{E3E4}$. Unlike the full-length transcript, $ZNF804A^{E3E4}$ is regulated in human fetal brain by the genome-wide psychosis risk allele rs1344706. This finding suggests that $ZNF804A^{E3E4}$ may be a critical isoform mediating the association and draws attention to the importance of alternative transcripts, and the fetal period, in the processes by which genetic variation affects neurodevelopmental psychiatric disorders. The fact that *ZNF804A* is translated into protein is also significant, increasing the likelihood that the gene has functional and pathophysiological roles in the brain. Equally, our data highlight the complexities involved when trying to identify the biological basis of a genetic association with a psychiatric disorder.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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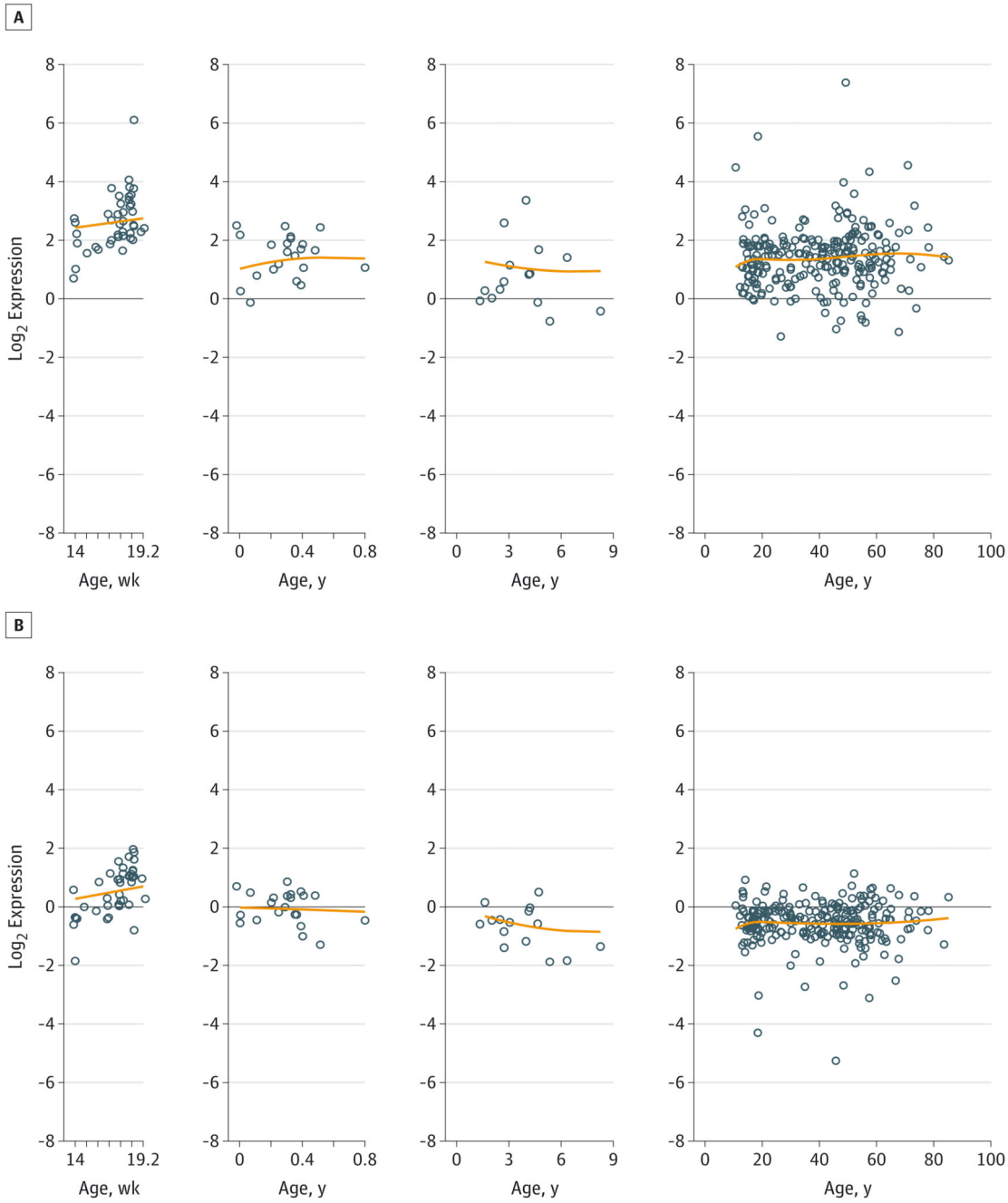

Figure 1. Expression Trajectories for *ZNF804A*^{E3E4} and *ZNF804A* Messenger RNA Across the Life Span

Life-span expression trajectories for the novel truncated transcript isoform *ZNF804A*^{E3E4} (A) and full-length *ZNF804A* (B) messenger RNA in human dorsolateral prefrontal cortex. Circles indicate observed data; lines, smoothing spline within each age stage.

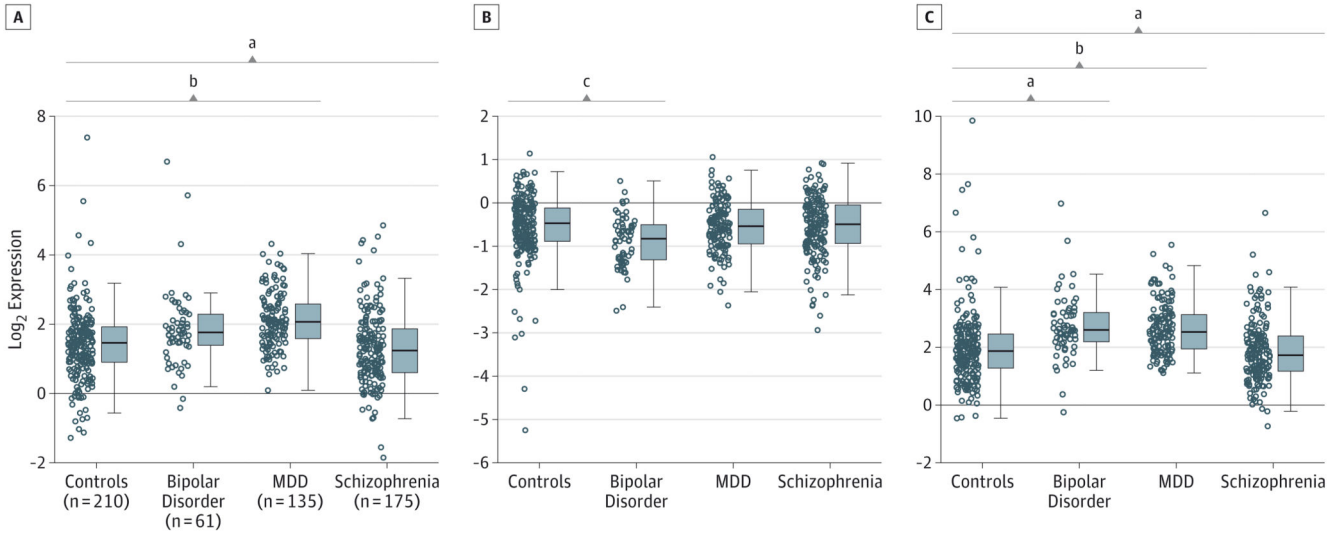

Figure 2. Diagnostic Effects on Expression of *ZNF804A* Transcripts in Dorsolateral Prefrontal Cortex

A, *ZNF804A*^{E3E4} messenger RNA (mRNA) shows elevated expression in major depressive disorder (MDD) but decreased expression in schizophrenia and no change in bipolar disorder compared with controls. B, Full-length *ZNF804A* mRNA shows decreased expression in bipolar disorder and no change in schizophrenia and MDD. C, Ratio of *ZNF804A*^{E3E4} to *ZNF804A* mRNA. Compared with controls, the ratio is greater (ie, relative increase in *ZNF804A*^{E3E4} mRNA) in bipolar disorder and MDD but lower in schizophrenia. Boxes indicate interquartile range; horizontal lines in boxes, median; and whiskers, 1.5 × interquartile range. The postmortem brain samples involved are from the National Institute of Mental Health/Lieber Institute for Brain Development series (Table).

[a] $P < .01$ for each diagnostic group vs controls in 1 linear model (see Methods).

[b] $P < .001$ for each diagnostic group vs controls in 1 linear model (see Methods).

[c] $P < .05$ for each diagnostic group vs controls in 1 linear model (see Methods).

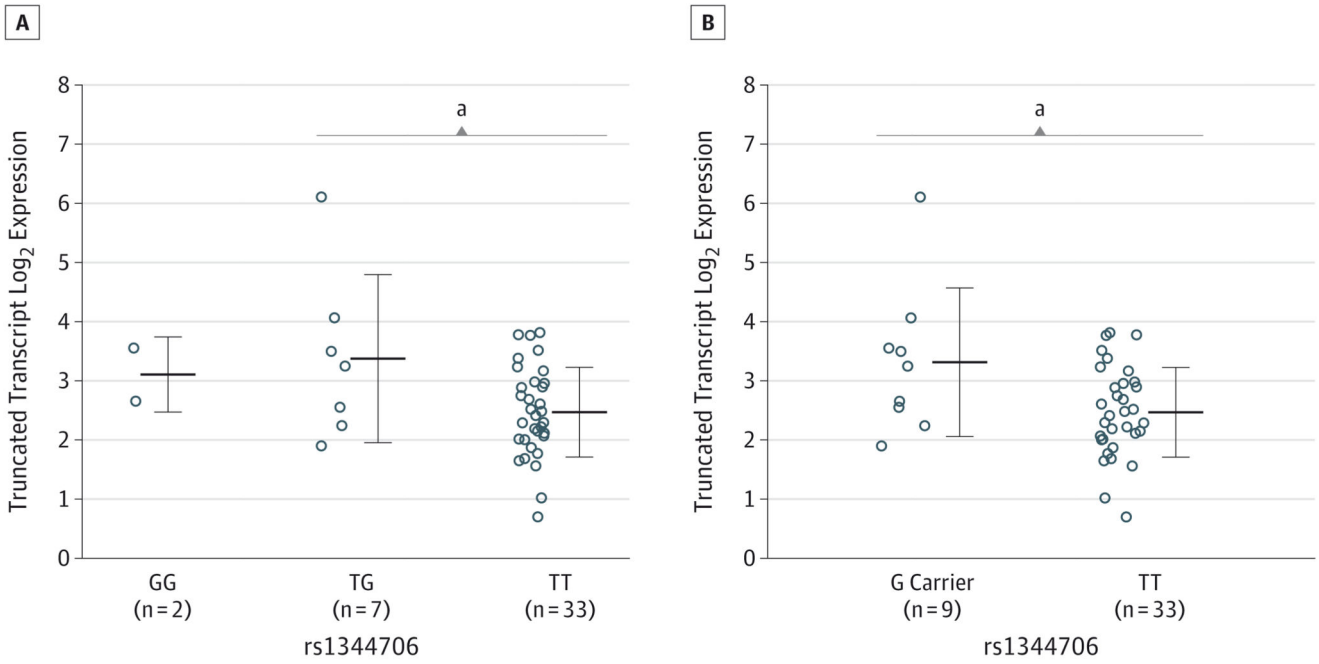

Figure 3. Genotype Effect of rs1344706 on *ZNF804A*^{E3E4} Messenger RNA in Human Fetal Brain

Homozygotes for the risk T allele are compared with GG homozygotes and TG heterozygotes (A) and with G carriers (B). Homozygotes for the risk T allele have lower expression than G carriers or TG heterozygotes. Center horizontal lines indicate mean; error bars, standard deviation.

[a] $P < .05$.

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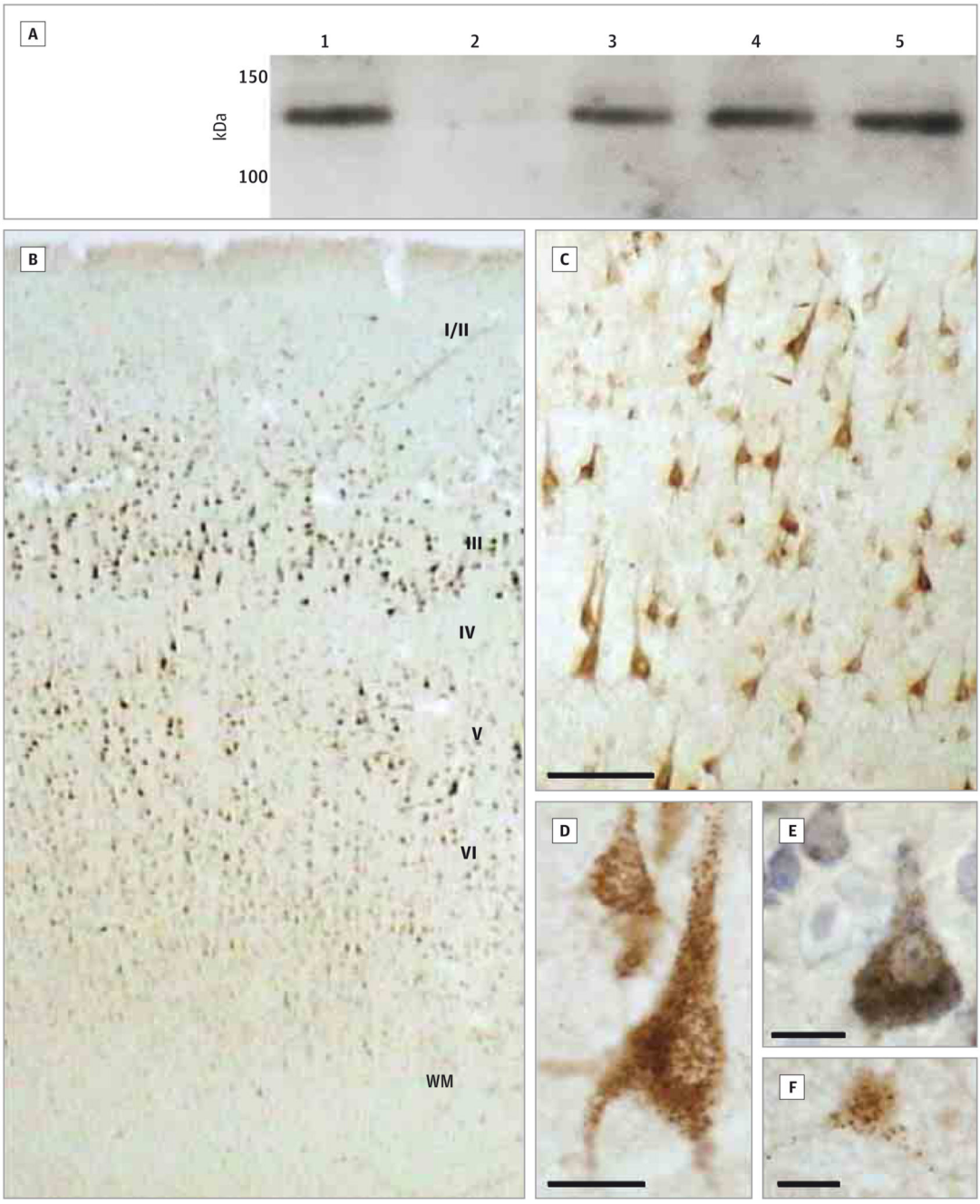

Figure 4. ZNF804A Immunoreactivity in Human Brain

A, Western blot. Lane 1 is HEK293 cells transfected with *ZNF804A*; lane 2, nontransfected HEK293 cells; lane 3, fetal cortex; and lanes 4 and 5, adult frontal cortex. eFigure 3 in the Supplement shows a full-length blot and the β-actin loading control, and eFigure 4 in the Supplement shows the D-14 blocking peptide control and a blot using a different anti-ZNF804A antibody. B, ZNF804A immunoreactivity through the inferior parietal cortex. Roman numerals indicate the cortical layers; WM, white matter. C, Layer III of the inferior parietal cortex (scale bar = 100 μm). D, Pyramidal neurons in layer III of the temporal cortex

(scale bar = 10 μm). E, Layer III of the inferior parietal cortex with Nissl counterstain (scale bar = 10 μm). F, Frontal cortex of a neonate (scale bar = 10 μm). eFigure 5 in the Supplement shows the immunohistochemistry peptide block control.

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Table

Participants Used for Studies of *ZNF804A* Transcripts in Dorsolateral Prefrontal Cortex

	Controls			Patients With Diagnosis		
Characteristic	**Fetal (n = 43)**	**Aged 0-18 y (n = 73)**	**Aged >18 y (n = 210)**	**Bipolar Disorder (n = 61)**	**Major Depressive Disorder (n = 135)**	**Schizophrenia (n = 175)**
Female, %	48.8[a]	32.8	29.0	41.0	42.2[a]	37.1
Age, mean, y	14-20[b]	8.6	44.2	44.8	45.7	50.2[a]
White, %[c]	11.6[a]	61.6[a]	41.4	83.6[a]	85.9[a]	54.3
pH	6.10[a]	6.43[a]	6.55	6.36[a]	6.35[a]	6.41[a]
RNA integrity number	8.81[a]	8.22	8.25	7.99[a]	8.03[a]	7.84[a]
Postmortem interval, h	2.6[a]	23.9[a]	30.7	32.9	37.9[a]	38.5[a]
Age at onset, mean, y	NA	NA	NA	23.0	31.1	22.4
Duration of illness, mean, y	NA	NA	NA	21.7	14.4	27.5

Abbreviation: NA, not applicable.

[a] $P < .05$ compared with adult controls.

[b] Expressed as range in gestational weeks.

[c] Assessment of race made by medical examiner and next of kin.