Molecular and Genetic Evidence for Abnormalities in the Nodes of Ranvier in Schizophrenia

Panos Roussos, MD, PhD; Pavel Katsel, PhD; Kenneth L. Davis, MD; Panos Bitsios, MD, PhD; Stella G. Giakoumaki, PhD; Jigar Jogia, PhD; Kinga Rozsnyai, MSc; David Collier, PhD; Sophia Frangou, MD, PhD; Larry J. Siever, MD; Vahram Haroutunian, PhD

Context: Genetic, neuroimaging, and molecular neurobiological evidence support the hypothesis that the disconnectivity syndrome in schizophrenia (SZ) could arise from failures of saltatory conduction and abnormalities at the nodes of Ranvier (NOR) interface where myelin and axons interact.

Objective: To identify abnormalities in the expression of oligodendroglial genes and proteins that participate in the formation, maintenance, and integrity of the NOR in SZ.

Design: The messenger RNA (mRNA) expression levels of multiple NOR genes were quantified in 2 independent postmortem brain cohorts of individuals with SZ, and generalizability to protein expression was confirmed. The effect of the ANK3 genotype on the mRNA expression level was tested in postmortem human brain. Case-control analysis tested the association of the ANK3 genotype with SZ. The ANK3 genotype’s influence on cognitive task performance and functional magnetic resonance imaging activation was tested in 2 independent cohorts of healthy individuals.

Setting: Research hospital.

Patients: Postmortem samples from patients with SZ and healthy controls were used for the brain expression study (n = 46) and the case-control analysis (n = 272). Healthy white men and women participated in the cognitive (n = 513) and neuroimaging (n = 52) studies.

Main Outcome Measures: The mRNA and protein levels in postmortem brain samples, genetic association with schizophrenia, cognitive performance, and blood oxygenation level–dependent functional magnetic resonance imaging.

Results: The mRNA expression of multiple NOR genes was decreased in schizophrenia. The ANK3 rs9804190 C allele was associated with lower ANK3 mRNA expression levels, higher risk for SZ in the case-control cohort, and poorer working memory and executive function performance and increased prefrontal activation during a working memory task in healthy individuals.

Conclusions: These results point to abnormalities in the expression of genes and protein associated with the integrity of the NOR and suggest them as substrates for the disconnectivity syndrome in SZ. The association of ANK3 with lower brain mRNA expression levels implicates a molecular mechanism for its genetic, clinical, and cognitive associations with SZ.


During the past decade, multiple lines of evidence (gene and protein expression, genetic association, neuroimaging) have suggested that the disconnectivity syndrome thought to underlie the neurobiology of schizophrenia (SZ) is in part due to abnormalities in myelination and oligodendroglial function. Efficient and fast neurotransmission is dependent on saltatory conduction, which is in turn dependent on the integrity of myelin sheaths, oligodendrocytes, and the nodes of Ranvier (NOR). The NOR span the entire length of myelinated axons and comprise myelin sheath–free segments where ion (sodium and potassium) exchange can take place, propagating action potentials from the axon initial segment down the length of the axon to synaptic terminals. In the central nervous system, NOR are formed and maintained through reciprocal interactions between neurons and oligodendrocytes. Multiple and specific neuronal and oligodendroglial proteins such as ankryrin G (AnkG) interact to ensure the high concentration (>1200 voltage-gated so-
dium ion channels/μm²) and anchoring of voltage-gated sodium ion channels (Nav; Nav1.6 in adults) to the NOR and maintain tight junctions between the axolemma and paranodal myelin loops. The AnkG protein belongs to a family of scaffolding proteins that interact with several proteins, including the cytoskeletal protein βIV spectrin and Nav channels. 5,6 The AnkG and Nav subunits also interact in cis with neurofascin 186 and paranodal contactin. 5,6 Contactin and Caspr interact in trans with cell adhesion molecules of oligodendroglial paranodal loops that include the neurofascin 155 isoform and Tag1 to ensure the integrity of the myelin-axolemma tight junction. 5,6 Abnormalities in the expression of oligodendrogial and neuronal genes and proteins in SZ could hamper salutary conduction by affecting the formation, maintenance, and integrity of the NOR and contribute to the hypothesized disconnectivity syndrome. 4

The current studies were undertaken to advance the observation of myelin and oligodendrocyte gene and protein expression abnormalities in SZ to the functional domain and to the study of genetic association based on direct neurobiological observations in the brains of persons with SZ with specific gene and protein expression abnormalities. Exploratory analysis of data from a large postmortem microarray study of multiple brain regions of persons with SZ 7,8 suggested the abnormal expression of many neuron- and oligodendrocyte-enriched genes that encode proteins associated with paranodal junctions and NOR. 5 We therefore sought to identify the involvement of axogial interacting genes and proteins in SZ more directly by using postmortem brain tissue from an independent cohort of persons with SZ and unaffected comparison controls.

ANK3 was among the genes that were abnormally expressed in independent gene and protein expression studies. Because recent genome-wide association studies have implicated ANK3 in bipolar disorder (BD) 10–13 and SZ 14 and because several studies have suggested commonalities in the neurobiology, 15–17 etiology, and genetic associations 12,13,18,19 between SZ and BD, we also sought to determine whether ANK3 genetic variants affected the expression of ANK3 in the same SZ and control brain specimens and in a larger postmortem collection of racially homogeneous cases and controls (n=272). The influence of the SZ risk–associated ANK3 polymorphism on cognitive domains of significance to SZ and on brain activity during performance of a working memory task was also examined in healthy controls (n=513 and n=52, respectively).

METHODS

SUBJECTS

Postmortem Cohort

Brain tissue specimens were derived from the Brain Bank of the Department of Psychiatry, Mount Sinai School of Medicine, New York, New York; James J. Peters Veterans Affairs Medical Center, Bronx, New York. The precise tissue-handling procedures have been described in detail. 20,21 The cause of death, type of antipsychotic medications used, mean age, postmortem inter-

val, tissue pH, and sex distributions of the subjects are shown in eTable 1 (http://www.archgenpsychiatry.com).

Gray matter from 14 cortical-frontal (Brodmann area [BA] 8, 10, 44, 46, 4), cingulate (BA 23/31, 24/32), parietal (BA 7), temporal (BA 20, 21, 22, 36/28, 38), and occipital (BA 17) brain regions and 3 noncortical brain regions (caudate, hippocampus, and putamen) were used for the Affymetrix HG-U133AB GeneChip gene expression analysis (n=17–39) (Affymetrix, Santa Clara, California) as described in detail previously. 22,24 Similarly prepared aliquots from the superior temporal gyrus (STG) and primary visual cortex were used in quantitative polymerase chain reaction (qPCR) and quantitative Western blotting analysis in a larger and independent set of brain tissue specimens (eTable 1). 23,26

Additional studies for genetic association of ANK3 rs9804190 were carried out using case-control samples from the Brain Bank. For analytical purposes, only white American individuals with Western European ancestry (SZ, n=208; comparison control, n=64) were used for the clinical genetic investigation of the ANK3 rs9804190 polymorphism.

Healthy Individuals

Learning on Genetics of Schizophrenia Spectrum Cohort and Cognitive Task Performance. We examined the effect of the ANK3 rs9804190 polymorphism using intermediates phenotypes in 530 individuals from the Learning On Genetics Of Schizophrenia Spectrum (LOGOS) cohort. This cohort has been described in detail previously. 27

British Cohort and Neuroimaging Data. We further explored the effect of the ANK3 rs9804190 polymorphism on brain activity (on functional magnetic resonance imaging [fMRI]) during a working memory task. Fifty-two healthy volunteers of white British descent were assessed with the Structured Clinical Interview for DSM-IV-TR Axis I Disorders and the Family Interview for Genetic Studies to exclude any personal psychiatric history or family history (up to second-degree relatives) of SZ or affective disorders. Participants were further screened to exclude past, current, and hereditary medical disorders, DSM-IV lifetime drug or alcohol dependence and drug or alcohol abuse in the preceding 6 months, and contraindications to MRI. For all participants, an estimate of the current full-scale IQ was obtained on the day of scanning using the Wechsler Adult Intelligence Scale–Revised and while handedness was based on self-report. Written informed consent was obtained from all subjects before study participation. The study was approved by the Joint Ethics Committee of the Institute of Psychiatry and the South London and Maudsley NHS Foundation Trust.

LONG-TERM HALOPERIDOL STUDIES IN RATS

To assess the effects of neuroleptic exposure on the expression of selected NOR genes of interest, groups of 8 male Sprague-Dawley rats (6–8 months of age) received daily subcutaneous injections of haloperidol (2 mg/kg) or the saline vehicle for 21 days. Ank3, Nfasc, Nrcam, and Scn8a expressions were assessed by the procedures described earlier using rat-specific primers and probes (eTable 2).

MICROARRAY PROCEDURE AND DATA ANALYSIS

Microarray analysis was performed using the Affymetrix HG-U133AB GeneChip set as described previously. 28 Statistical com-
parisons were made using the GeneSpring GX 7.3.1 cross-
gene error model (Agilent Technologies/Silicon Genetics, Santa
Clara), based on the deviation from 1.0 algorithm. Filtering con-
ditions were a combination of confidence (P ≤ .05) and fold
change (≥1.6) with Benjamini and Hochberg13 multiple test-
ing corrections.

**Real-time qPCR**

The messenger RNA (mRNA) levels of selective NOR genes were
measured by qPCR in a larger independent (from the micro-
array cases and controls) cohort (eTable 1) using TaqMan probes
and primer sets (Applied Biosystems, Foster City, California).
TaqMan probe identification numbers for selected nodal genes
and their unique probes are listed in eTable 2. For relative
quantification of mRNA expression, geometric means of the
expression of 3 housekeeping genes were calculated using the
standard curve method. The housekeeping genes PGK1, PPIA, and
RPLPO were selected for their stability after comparison of sev-
eral different endogenous controls using geNorm (http://medgen.
ugent.be/~jvdesomp/genorm/). Two housekeeping genes (GADPH
and PPIA) were used as the endogenous references in the rat
studies.

**QUANTITATIVE WESTERN BLOTTING**

Protein abundance was measured in the STG from patients with
SZ and comparison control subjects (n=8/group) previously
selected for qPCR analysis using Western blotting. Blots were
probed with mouse monoclonal anti-AnkG antibody (IgG1, 1:200
vol/vol dilution; NeuroMab, Davis, California), which binds spec-
difically to 2 different isoforms (approximately 270 kDa and
approximately 250 kDa) and rabbit anti–glyceraldehyde-3-
phosphate dehydrogenase antibody (1:5000 vol/vol dilution;
NeuroMab, Davis, California), which binds specifically to 2
different isoforms (approximately 270 kDa and 70 kDa; slice
thickness, 7 mm; slice width, 0.7 mm; matrix size, 64 × 64;
voxel dimensions, 3.75 × 3.75 × 7.7 mm). For the pur-
pose of anatomical localization, a T1-weighted 3-dimen-
sional inversion recovery prepared spoiled gradient recalled se-
quence was used to acquire structural images in 124 axial planes
(repetition time, 1800 milliseconds; echo time, 5.1 milli-
seconds; inversion time, 450 milliseconds; flip angle, 20°; slice thic-
ness, 1.3 mm; matrix size, 256 × 192; field of view, 240 × 180
mm; voxel dimensions, 0.9375 × 0.9375 × 1.5 mm; number of ex-
citations, 1).

**THE N-BACK TASK IN NEUROIMAGING**

The 3-back task was administered in a block design. Partici-
pants were instructed to respond by button press to the target
letter. In the baseline (0-back) condition, the designated tar-
get letter was X. In the experimental condition (3-back), the
target letter was defined as any letter that was identical to the
one presented 3 trials back. In each condition, a series of 14
letters were visually presented for 2 seconds each. Responses
were monitored via a magnetic resonance–compatible button
box held in the subject's dominant hand. There were 12 ep-
ochs in all, each lasting 30 seconds, with a total experiment
duration of 6 minutes. The ratio of target to nontarget letters
presented per block ranged from 2:12 to 3:11. Reaction time to
target letters and number of correct responses (accuracy) were
recorded.

**IMAGE ACQUISITION**

Both anatomical and gradient-echo echoplanar imaging data were
acquired during the same session using a 1.5-T neuro-
optimized Signa MRI system (General Electric, Milwaukee, Wis-
consin). A total of 150 T2*-weighted echoplanar imaging brain
volumes depicting blood oxygenation level–dependent con-
trast were acquired at each of the 16 axial planes (repetition
time, 2000 milliseconds; echo time, 40 milliseconds; flip angle,
70°; slice thickness, 7 mm; slice width, 0.7 mm; matrix size,
64 × 64; voxel dimensions, 3.75 × 3.75 × 7.7 mm). For the
purposes of anatomical localization, a T1-weighted 3-dimen-
sional inversion recovery prepared spoiled gradient recalled se-
quence was used to acquire structural images in 124 axial planes
(repetition time, 1800 milliseconds; echo time, 5.1 milli-
seconds; inversion time, 450 milliseconds; flip angle, 20°; slice thic-
ness, 1.3 mm; matrix size, 256 × 192; field of view, 240 × 180
mm; voxel dimensions, 0.9375 × 0.9375 × 1.5 mm; number of ex-
citations, 1).

**DNA GENOTYPING AND CLINICAL ASSOCIATION ANALYSIS**

Samples of DNA from the postmortem cohort were extracted
from the STG using the Genomic DNA-Tissue MiniPrep kit
(Invitrogen, Irvine, California). We initially genotyped the
qPCR cohort (n=48) for ANK3 rs9804190, rs10994336, and
rs10761482 polymorphisms that contribute to SZ and BD
risk.1114 (Polymorphic DNA Technologies, Alameda, Califor-
nia). Genotyping of the LOGOS cohort was performed blind
to target letters and number of correct responses (accuracy) were
recorded.

**STATISTICAL ANALYSIS**

Multiple statistical procedures were used for different aspects of
the study. Comparison of the demographic variables was per-
formed using separate 1-way analyses of variance or the non-
parametric Mann-Whitney U test as appropriate, based on de-
viation from normality. A 2-tailed t test was used to compare
the relative abundance of AnkG protein and the relative ANK3
mRNA expression of NOR genes in qPCR and genotyping ex-
periments using SPSS version 17 statistical software (SPSS Inc,
Chicago, Illinois).

We used QTPHASE software (http://www.mrc-bsu.cam.ac.
.uk/personal/frank/software/unphased/) from the UNPHASED
package version 3.1.4 for the analysis of genotype associa-
tions in the LOGOS cohort and ANK3 expression levels in the
qPCR cohort. The ANK3 rs9804190 polymorphism was exam-
ined for allelic association with SZ in the Brain Bank cohort
with PLINK software.33 (http://pngu.mgh.harvard.edu/~purcell/
/plink/). Quality control for the Brain Bank cohort was done as
described previously.15,16 correction for population stratifica-
tion was achieved using the eigenstrat method.34 Logistic re-
gression analysis was carried out to evaluate the association
of rs9804190 with SZ. P values from the UNPHASED and PLINK
analyses were corrected for multiple testing by running 100,000
permutations of the data. Additionally, to lower the possibil-
ity of type I statistical error in the LOGOS analysis, we set
the level of significance to .01.

For the IMRI data analysis, we used the Statistical Parametric
Mapping (SPM5) software (http://www.fil.ion.ucl.ac.uk/
/spm/software/spm5) implemented in MATLAB 7.1 (MathWorks

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Table 1. Microarray-Based Gene Expression Analysis of Selected Nodal and Tight Junction Genes

<table>
<thead>
<tr>
<th>Sequence Cluster</th>
<th>Gene Symbol</th>
<th>Maximum t Score</th>
<th>P Value</th>
<th>FC Ratio</th>
<th>Gene Symbol</th>
<th>Maximum t Score</th>
<th>P Value</th>
<th>FC Ratio</th>
<th>Gene Symbol</th>
<th>Maximum t Score</th>
<th>P Value</th>
<th>FC Ratio</th>
<th>Gene Ontology Biological Process</th>
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<tr>
<td>Unregulated in SZ</td>
<td>SCN1B</td>
<td>5.23</td>
<td>.06</td>
<td>1.09</td>
<td>.18</td>
<td>1.28</td>
<td>Sodium ion transport</td>
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<td></td>
<td>SCN3B</td>
<td>4.94</td>
<td>.42</td>
<td>1.15</td>
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<td>KCNAB1</td>
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<td>Cation transport</td>
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<td></td>
<td>KCHN7</td>
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<td></td>
<td>CNTNAP2</td>
<td>3.06</td>
<td>.01</td>
<td>1.45</td>
<td>.31</td>
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<td>Cell adhesion</td>
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<td>Downregulated in SZ</td>
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<td>MAG</td>
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<td>TAG1</td>
<td>−5.43</td>
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<td>1.52</td>
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<td>1.07</td>
<td>Cell-cell adhesion</td>
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<td>NRCAM</td>
<td>−2.52</td>
<td>.1</td>
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<td>.61</td>
<td>1.07</td>
<td>Cell-cell adhesion</td>
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<td></td>
<td>TJP1</td>
<td>−2.09</td>
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<td>1.13</td>
<td>.53</td>
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<td>Interacellular junction assembly</td>
<td></td>
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</table>

Abbreviations: FC, fold change; PVC, primary visual cortex; STG, superior temporal gyrus; SZ, schizophrenia.

*Data represent aggregate expression change scores across 17 different brain regions. Sample sizes vary for the different brain regions analyzed. The maximum t score is an extension of the fold change algorithm that was used as a standardized measure of gene expression change in SZ across all analyzed brain regions. Analysis of variance P < .05 for all of the listed genes. Upregulated and downregulated genes are listed in descending order of change score. The FC ratios and P values are from the same microarray collection separately for the STG and PVC.

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RESULTS

GENE EXPRESSION OF NOR GENES IS AFFECTED IN SZ

We initially conducted an exploratory analysis using microarray data (contrast analysis t scores) from 17 different brain regions representing all 4 cortical lobes, the hippocampus, and basal ganglia in persons with SZ (n=9-21) vs unaffected comparison controls (n=8-18) (eTable 1). We found significant dysregulation in the expression of genes known to participate in the development, organization, and maintenance of the NOR across all brain regions (Table 1). The majority of the genes, classified by gene ontology analysis as associated with cell adhesion, were downregulated in the assayed brain regions of persons with SZ.

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Eight genes known to be involved in the organization and maintenance of NOR were selected from those shown in Table 1 for qPCR analysis in an independent and larger sample of well-matched cases (n = 22) and controls (n = 24). The expression of these genes was assessed in the STG and primary visual cortex (eTable 1 and eTable 2), brain regions where we found significant and minimal alterations of genes expression, respectively, in our microarray analysis (Table 1). Other relevant genes (eg, Nav1.6) that were expressed at too low a level for detection by microarray were also included in this analysis. As shown in Table 2, the STG of this independent cohort of persons with SZ, evidenced significantly reduced mRNA expression of ANK3 (P = .01; fold change [FC] = −1.25) (Figure 1), neurofascin (NFASC) (P = .02; FC = −1.33), neuronal cell adhesion molecule (NRCAM) (P = .01; FC = −1.3), and Nav1.6 sodium channel, α subunit (SCN8A) (P = .04; FC = −1.34) and a trend for contactin 2 (CNTN2 or TAG1) (P = .08; FC = −1.3).

These changes were gene specific given that the mRNA levels of other nodal proteins, including contactin 1 (CNTN1), contactin-associated protein-like 2 (CNT-NAP2), or Nav1.2 sodium channel, α subunit (SCN2A), did not show significant differences among persons with SZ and comparison controls (all P > .19). Brain region specificity was evident from the lack of changes in any of these genes in the primary visual cortex (all P > .12). Sample pH, postmortem interval, or age of the donors did not alter the outcome of the analysis when entered into statistical analyses as covariates.

All of the persons with SZ included in these studies had received antipsychotic medications for many years. To test whether the expression of axoglial junction genes is affected by the classic and most frequently prescribed antipsychotic medication in this cohort, the expression levels of Ank3, Nrcam, NFasc, and Scn8a were measured.

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Table 2. Gene Expression Analysis of Selected Nodes of Ranvier Genes in the Superior Temporal Gyrus and Primary Visual Cortex<sup>a</sup>

<table>
<thead>
<tr>
<th>Gene Name (Symbol)</th>
<th>STG P Value</th>
<th>FC Ratio</th>
<th>PVC P Value</th>
<th>FC Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurofascin (NFASC)</td>
<td>.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−1.33</td>
<td>.76</td>
<td>−1.05</td>
</tr>
<tr>
<td>Contactin 2 (CNTN2/TAG1)</td>
<td>.08</td>
<td>−1.30</td>
<td>.37</td>
<td>−1.27</td>
</tr>
<tr>
<td>Ankyrin 3, nodes of Ranvier (ANK3)</td>
<td>.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−1.25</td>
<td>.23</td>
<td>−1.11</td>
</tr>
<tr>
<td>Contactin 1 (CNTN1)</td>
<td>.30</td>
<td>−1.09</td>
<td>.76</td>
<td>1.03</td>
</tr>
<tr>
<td>Contactin-associated protein-like 2 (CASPR2)</td>
<td>.19</td>
<td>−1.23</td>
<td>.12</td>
<td>−1.25</td>
</tr>
<tr>
<td>Neuronal cell adhesion molecule (NRCAM)</td>
<td>.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−1.30</td>
<td>.51</td>
<td>1.09</td>
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<td>Tenascin-R (TNR)</td>
<td>.01</td>
<td>−1.19</td>
<td>.56</td>
<td>1.13</td>
</tr>
<tr>
<td>Gliomedin (GLDN)</td>
<td>.24</td>
<td>−1.23</td>
<td>.77</td>
<td>−1.06</td>
</tr>
<tr>
<td>Sodium channel, voltage-gated, type II, α subunit, Nav1.2 (SCN2A)</td>
<td>.70</td>
<td>−1.03</td>
<td>.79</td>
<td>−1.02</td>
</tr>
<tr>
<td>Sodium channel, voltage-gated, type VIII, α subunit, Nav1.6 (SCN8A)</td>
<td>.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−1.34</td>
<td>.16</td>
<td>−1.24</td>
</tr>
</tbody>
</table>

Abbreviations: FC, fold change; PVC, primary visual cortex; STG, superior temporal gyrus.

<sup>a</sup>Tissue samples from the STG and PVC of control subjects (n = 24) and persons with schizophrenia (n = 22) were analyzed by quantitative polymerase chain reaction. The FC ratios represent the ratio of the geometric means for each gene to the 3 housekeeping genes in SZ/control values (positive) and in control/SZ values (negative).<sup>b</sup> Differently changed genes (P < .05).

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Figure 1. ANK3 gene expression and protein levels are significantly decreased in the superior temporal gyrus of patients with schizophrenia (SZ) compared with control subjects. A, Gene expression of ANK3 is significantly decreased in the superior temporal gyrus of persons with SZ vs control subjects (P = .01; fold change = −1.25). The ANK3 rs8041990-associated differences show reduced expression of the ANK3 transcript in persons with SZ homozygous for the C allele in comparison with the T allele carriers (P = .03). Two subjects were not successfully genotyped. Data are expressed as mean ± SEM of individual expression values normalized to the geometric mean of 3 housekeeping genes: PGK1, PPIA, and RPLPO. B, The protein level of the AnkG 270-kDa isoform is significantly decreased in the superior temporal gyrus of patients with SZ compared with control subjects (n = 8 per group). The anti-AnkG antibody binds also to a less characterized AnkG 250-kDa isoform, which was also downregulated in patients with SZ (P < .02). The individual AnkG expression values shown are normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Horizontal lines indicate the group means.
in the cerebral cortex of rats receiving long-term treatment with haloperidol and compared with saline vehicle-treated controls. Three weeks of relatively high-dose (2 mg/kg/d) treatment with haloperidol did not influence the cerebral cortical expression of the examined nodal genes (all P > .50).

Based on the multiple lines of evidence described earlier supporting decreased ANK3 mRNA expression levels, the protein levels of AnkG in STG were measured in a subset of individuals with SZ (n = 8) vs comparison subjects (n = 8) well matched for age, sex, pH, and postmortem interval from the larger set of subjects used in the qPCR studies described earlier (eTable 1). As shown in Figure 1, the levels of AnkG protein were significantly decreased (P = .03) in the STG of persons with SZ relative to controls.

**ANK3 rs9804190 C Allele Increases Risk for SZ by Affecting ANK3 Expression Levels**

Given the association of ANK3 with BD10-13 and SZ14 in genetic studies and the evidence of ANK3 gene and AnkG protein expression deficits in the brain of persons with SZ, we determined whether ANK3 genetic variants are associated with ANK3 gene expression in the STG. Because of the low frequency of minor (T) allele homozygotes, carriers of the minor allele (C/T and T/T) were grouped together. A significant main effect of genotype was observed for rs9804190 in the STG with ANK3 gene expression in individuals with SZ (P = .03). Among persons with SZ, those who were homozygous for the C allele had approximately 27% lower levels of ANK3 expression compared with individuals with at least 1 minor T allele (Figure 1). The UNPHASED analysis revealed that the rs9804190 C allele was associated significantly (P = .006) with lower ANK3 expression in the STG of individuals with SZ; the estimated additive genetic value between the C and T alleles was −4.17. A significant effect of the C/C allele in controls was not found (P = .82). No significant effect was observed for other ANK3-associated single-nucleotide polymorphisms,10-13 rs10994336 or rs10761482, in the STG (all P > .18) or any of the ANK3 genotypes in the primary visual cortex (all P > .13).

Based on the association of the ANK3 rs9804190 C allele with lower ANK3 expression levels and the finding of decreased ANK3 expression in SZ, the association of the rs9804190 C allele, which increases the risk for SZ, was examined in a case-control study. Using a larger postmortem cohort of white persons with SZ (n = 208) relative to controls (n = 64), the study revealed a significant association of the ANK3 rs9804190 C allele with SZ (permeuted P = .03; odds ratio = 1.77; 95% confidence interval, 1.08-2.88).

We further examined whether the ANK3 rs9804190 C allele is associated with poorer performance in SZ-related behavioral assays. Therefore, the association of the rs9804190 C allele with performance on a test of cognition sensitive to SZ was investigated in 513 graphically homogeneous, healthy, young, white individuals without psychopathology (LOGOS cohort27). There were no differences in demographic characteristics based on ANK3 rs9804190 genotype (eTable 4). Table 3 shows the association of the ANK3 rs9804190 polymorphism with a broad array of phenotypic cognitive function measures as revealed by QTPHASE analysis. The rs9804190 C allele was associated with statistically significant (P < .01) lower correct responses on the 2-back and 3-back conditions of the N-back sequential letter task, fewer completed categories and an increased number of total and perseverative errors during the Wisconsin Card Sorting Test, and lower self-directedness and higher self-transcendence scores on the Temperament and Character Inventory. Results were not altered when age, education, smoking status (cigarettes per day), and IQ were entered as covariates. There were no significant associations with baseline acoustic startle, prepulse inhibition of the startle response, and other cognitive tasks or personality dimensions, with or without covarying for age, IQ, and smoking status (cognitive tests and prepulse inhibition: all P > .05).

The final experiment used blood oxygenation level–dependent fMRI to examine the effect of the ANK3 rs9804190 polymorphism in an independent cohort of healthy volunteers (n = 52). Because of the small number of TT homozygotes, all carriers of the T variant were grouped together for further analysis. Homozygotes of the ANK3 rs9804190 risk C allele compared with the T allele carriers showed increased activation (as measured by fMRI) in 2 prefrontal clusters centered in the left inferior frontal gyrus (BA 11/47; x = −36, y = 40, z = −2; voxels = 218; z score = 3.34) and the left middle frontal gyrus (BA 45/46; x = −46, y = 30, z = 20; voxels = 114; z score = 3.15) during performance of the 3-back version of the N-back working memory task (Figure 2). There was no effect of genotype on demographic or task performance variables or IQ (all P > .53) (eTable 5).

**COMMENT**

These results provide insights into the mechanistic processes and genetic substrates associated with the frequently observed myelin- and oligodendrocyte-related deficits in SZ.1,2 They point to an abnormality in the expression of genes and proteins associated with the integrity of the NOR and by extension with the efficiency of neurotransmission. The gene expression results are strengthened by replication in multiple cohorts and diverse methods and by the observation of a novel genetic association of at least 1 of the key proteins of the NOR, AnkG, with SZ and with cognitive functions known to be impaired in SZ. Although the ANK3 single-nucleotide polymorphism is within a noncoding region of the ANK3 gene, the gene expression findings indicate an association not only with SZ but also with reduced gene expression in the STG. The genetic association findings are strengthened by their consistency across multiple study groups and by the fact that the ANK3 genetic association with SZ is also known to be associated with increased risk for BD.10-13 There is evidence suggestive of etiological convergence between SZ and BD12,16,19 consistent with these results.

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The molecular mechanism(s) of the ANK3 genetic susceptibility for SZ contributes to reduced gene and protein expression of ANK3/AnkG, with disease-relevant anatomic specificity. The expression of ANK3 and other nodal genes is reduced in the STG, a brain region that has been repeatedly shown to be vulnerable in SZ, but not in the visual cortex where SZ-dependent abnormalities are rarely observed. The current observations derived from a specific evidence-based hypothesis testing approach: preliminary findings from multiregional microarray expression studies of oligodendrocyte- and myelin-associated deficits in SZ led to hypothesized abnormalities of the NOR. This hypothesis was tested and supported by independent gene and protein expression experiments. The observation of abnormal expression of known NOR-enriched genes and proteins led directly to genetic association studies and linkage of genetic association with cognitive, behavioral, and neuroimaging intermediate phenotypes repeatedly linked to SZ.

The mRNA expressions of the NOR-enriched proteins ANK3, NFASC, NRCAM, SCN8A, and CNTN2 were all significantly downregulated in the STG of persons with SZ. Increasing evidence points to a pivotal role of AnkG in the initial assembly and concentration of Nav channels and cell adhesion molecules to central nervous system NOR. In addition, a significant role for cell adhesion molecules in Nav channels clustering has been demonstrated in in vitro experiments where treatments with antibodies against the cell adhesion molecules lead to a failure of both Nav channels and AnkG to accumulate at the NOR. That many of these proteins demonstrated to be crucial to the normal functioning of the NOR and to salutatory conduction are abnormally expressed in SZ and the genetic linkage of ANK3 with SZ attest to their importance to the neurobiology of the disease. However, despite significant recent advances, the mechanisms of formation and maintenance of the NOR remain poorly understood and the full complement of known genes and proteins that contribute to their assembly, maintenance, and function is still incomplete. One speculative mechanism is that NOR abnormalities will affect the conduction velocity, leading to attenuated, slow, abnormally variable or absent action potentials. Indeed, in an ANK3 knockout mouse model, the generation and persistence of action potentials were dramatically inhibited. Such abnormalities will limit the distance over which cortical neurons can fire in synchrony, affecting the normal long-range oscillations between cortical subfields that could lead to attention deficits.

AnkG and many of the proteins whose mRNA expressions were studied here are not exclusively localized to the NOR. AnkG is also essential for the localization of many membrane proteins to the axon initial segment, including the Nav channels that are required for action potential generation. "Cruz et al showed that the density of the AnkG-immunoreactive axon initial segment in subjects with SZ was significantly reduced in superficial, but not in deep, cortical layers compared with both healthy comparison subjects and subjects with major depressive disorder. This might provide an additional mechanism in which AnkG and Nav clustering abnormalities impair action potential generation, disrupting neurotransmission and the capacity of some neurons to fire in the repetitive and synchronous fashion required for cortical network oscillations.

While the ANK3 single-nucleotide polymorphisms identified are within noncoding regions of the gene, they nevertheless support a role for ANK3 in susceptibility to SZ and performance in cognitive measures. Although our
strategy of a deductive, direct, postmortem neurobiology study minimized serendipitous findings, it did involve multiple statistical tests. Thus, the potential for spurious associations because of multiple testing is an important consideration. However, a consistent pattern of ANK3 rs9804190 association was found in healthy subjects, involving cognition and functional imaging, and in ANK3 gene and protein expression levels in brain tissue. The likelihood that the same ANK3 risk allele was consistently associated with illness or with illness-associated abnormalities across multiple geographically diverse samples by chance seems small.

ANK3 has recently been shown to be a susceptibility gene for BD, further supporting the notion of shared genetic vulnerabilities between BD and SZ. However, to our knowledge, there has been no previous report of an association of the ANK3 rs9804190 variant with SZ. This may reflect the fact that *P* values reported in this a priori hypothesis–driven postmortem brain-based genetic association study would not be significant after genome-wide association study correction for hypothesis-free multiple testing.

The absence of an ANK3 genetic variant effect on ANK3 mRNA expression levels in healthy individuals might be due to a lack of power. In patients with SZ, additive effects such as epistasis or environmental factors might augment the effect size of ANK3 genetic variants. We identified an effect of the rs9804190 C allele in a larger population of healthy subjects (LOGOS cohort) and using a sensitive approach (neuroimaging cohort), further supporting the notion of inadequate power.

Antipsychotic medication exposure is a confounding factor in postmortem studies of SZ and therefore can contribute to the detected gene expression abnormalities of the axoglial genes. We did not find an altered expression level in the cerebral cortex of rats treated with haloperidol. However, the possibility of antipsychotic medication exposure confounds cannot be entirely ruled out, as haloperidol exposure in rats might not exactly mimic the neuroleptic-induced changes in the brain of patients with SZ. Nevertheless, never-medicated, healthy, and asymptomatic ANK3 risk allele carriers present with poorer working memory and executive function, indices of reduced functionality (self-directedness), and increased prefrontal activity during a working memory task. These findings indicate a penetrant mechanism of the ANK3 gene in behavior that might increase the risk for SZ through poor cognition. Collectively, these findings identify SZ–associated abnormalities in NOR genes and proteins and provide significant evidence for neurobiological mechanisms and functions that might be key to the pathophysiology of SZ and to the development of targeted therapeutic interventions.

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Author Affiliations: Department of Psychiatry, The Mount Sinai School of Medicine, New York (Drs Roussos, Katz, Davis, Siever, and Haroutunian), and James J. Peters Veterans Affairs Medical Center, Bronx (Drs Roussos, Siever, and Haroutunian), New York; Department of Psychiatry and Behavioral Sciences, Faculty of Medicine, University of Crete, Heraklion, Crete, Greece (Drs Roussos, Bitsios, and Giakoumaki); and Section of Neuropathology of Psychosis (Drs Jogia and Frangou) and Social, Genetic, and Developmental Psychiatry Research Centre (Ms Rosznyai and Dr Collier), Institute of Psychiatry, King’s College London, England.

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Correspondence: Vahram Haroutunian, PhD, Department of Psychiatry, The Mount Sinai School of Medicine, Room 4F-33, 130 W Kingsbridge Rd, Bronx, NY 10468 (vahram.haroutunian@mssm.edu).

Author Contributions: Drs Roussos and Katsel contributed equally to this work. Drs Roussos and Haroutunian had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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