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

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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.1-3 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).4 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.5 Multiple and specific neuronal and oligodendroglial proteins such as ankyrin G ( AnkG) interact to ensure the high concentration (>1200 voltage-gated so-
eral proteins, including the cytoskeletal protein BIV spectrin and Nav channels.5,6 The AnkG protein belongs to a family of scaffolding proteins that interact with several proteins, including the cytoskeletal protein BIV 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 oligodendroglial and neuronal genes and proteins in SZ could hamper saltatory 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 oligodendroglial 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 SZ27,28 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 axoglial 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)10,11 and SZ14 and because several studies have suggested commonalities in the neurobiology,15-17 etiology, and genetic associations12,13,18,19 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.20-22 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.23,24 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).25,26

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 Disorders28 and the Family Interview for Genetic Studies29 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,30 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.79 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-
tions were a combination of confidence (P<.05) and fold change (≥1.6) with Benjamini and Hochberg11 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 primers used in qPCR in the qPCR cohort are listed in Table 2. For relative quantification of mRNA expression, geometric means of the ex-
pression of 3 housekeeping genes were calculated using the stan-
dard curve method. The housekeeping genes PGK1, PPIA, and
RPLP0 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 (lgG2, 1:200
vol/vol dilution; NeuroMab, Davis, California), which binds spe-
cifically to 2 different isoforms (approximately 270 kDa and
approximately 230 kDa) and rabbit anti–glyceraldehyde-3-
phosphate dehydrogenase antibody (1:5000 vol/vol dilution;
LifeSpan Biosciences, Seattle, Washington). Visualization and
quantification of bands were performed with the Odyssey 2.1
software (Li-COR Biosciences, Lincoln, Nebraska). To ac-
count for gel-to-gel variability, the relative expression value of
AnkG and glyceraldehyde-3-phosphate dehydrogenase in each
sample was calculated as a ratio between the averaged inten-
sities of the band in the experimental sample and in the stan-
dard calibrator (a mix of small aliquots of tissue from all
samples).

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
(Zymo Research, Irvine, California). We initially genotyped the
qPCR cohort (n=48) for ANK3 rs9804190, rs10994336, and
rs10761482 polymorphisms that contribute to SZ and BD
risk.10-11,14 (Polygenic DNA Technologies, Alameda, Califor-
ia). Genotyping of the LOGOS cohort was performed blind to
phenotype measures with a competitive allele-specific PCR
system (KBioscience, Herts, England). Brain Bank cohort geno-
types were analyzed by Children’s Hospital of Philadelphia, Phila-
delphia, Pennsylvania, using the Affymetrix single-nucleotide
polymorphism 6.0 array. Genotyping of the British cohort was
determined by a TaqMan allelic discrimination assay (Applied
Biosystems, assay C_2584015_10). Genotyping quality control
for ANK3 rs9804190 in both the Brain Bank and LOGOS
cohorts was performed in more than 10% of the samples by dup-
licate checking (rate of concordance in duplicates >99%). Call
rate was approximately 97% for all polymorphisms. The mi-
nor allele frequencies of the ANK3 rs9804190 variant were ident-
cal across the different cohorts (eTable 3).

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 each, 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,
Wisconsin). 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 skip, 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
thickness, 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-
icitations, 1).

STATISTICAL ANALYSIS

Multiple statistical procedures were used for different aspects of
the study. Comparison of the demographic variables was per-
cformed 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
experiments using SPSS version 17 statistical software (SPSS Inc,
Chicago, Illinois).

We used QTPHASE software (http://www.mrc-bsu.cam.
.uk/personal/frank/software/unphased/) from the UNPHASED
package32 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://pmg.mgh.harvard.edu/~purcell /
/plink/). Quality control for the Brain Bank cohort was done as
described previously35; correction for population stratifica-
tion was achieved using the eigenstrat method.34 Logistic
regression 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 1 statistical error in the LOGOS analysis, we set
the level of significance to .01.
PVC

ratios and considered significant at analysis (group 1: CC; group 2: CT and TT). Results were con-

estimates) from the suprathreshold cluster were extracted with

maxima of the suprathreshold clusters were converted from the

multiple comparisons. Stereotactic coordinates of the peak

and entered in the second-level, random-effects, 2-sample

3-back vs 0-back conditions were produced for each subject

response function–shaped low-pass filter was used to filter low-

applied to remove low-frequency noise. A hemodynamic re-

the vectors of interest and a high-pass filter (128 seconds) was

computed for each participant. The fMRI responses were con-

smoothing with an 8-mm full-width half-maximum isotropic

ture space using the participant's structural MRI images, and

movement, normalization into Montreal Neurological Insti-

Preprocessing of the images involved realignment to correct for

Inc, Natick, Massachusetts) for data processing and analysis.

Preprocessing of the images involved realignment to correct for

movement, normalization into Montreal Neurological Insti-

tute space using the participant's structural MRI images, and

smoothing with an 8-mm full-width half-maximum isotropic gaussian kernel. A first-level fixed-effects model was computed for each participant. The fMRI responses were convolved with a canonical hemodynamic response function with

a canonical hemodynamic response function with

member 7

Sodium channel, voltage-gated, type X, α

SCN10A

3.36
<.01
1.74
.51
−1.13
Cation transport

Downregulated in SZ

Neurofascin

NFASC

−8.12
.04
−1.26
.27
−1.10
Cell adhesion

Claudin 11

CLDN11

−7.29
.009
−1.68
.18
−1.61
Calcium-independent cell-cell adhesion

Myelin-associated glycoprotein

MAG

−7.24
.02
−1.28
.93
−1.03
Cell adhesion

Tight junction protein 2

TJP2

−6.35
.02
−1.54
.15
−1.39
Intercellular junction assembly

Contactin 2

CNTN2

−6.10
.52
−1.13
.49
−1.11
Cell adhesion

Ankyrin G

ANK3

−5.43
.001
−1.52
.23
1.16
Cytoskeletal anchoring

Contactin 3

CNTN3

−2.53
.01
−1.41
.13
−1.33
Cell adhesion

Neuronal cell adhesion molecule

NRCAM

−2.52
.31
−1.13
.61
1.07
Cell-cell adhesion

Tight junction protein 1

TJP1

−2.09
.24
−1.13
.53
−1.08
Intercellular junction assembly

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 < .01 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.

b P < .05.

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.
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 (CNTNAP2), 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.

| Table 2. Gene Expression Analysis of Selected Nodes of Ranvier Genes in the Superior Temporal Gyrus and Primary Visual Cortexa |
|--------------------|----------------|-----------------|-----------------|----------------|
| Gene Name (Symbol) | STG Value FC Ratio | PVC Value FC Ratio |
| Neurofascin (NFASC) | .02 | −1.33 | .76 | −1.05 |
| Contactin 2 (CNTN2/TAG1) | .08 | −1.30 | .37 | −1.27 |
| Ankyrin 3, nodes of Ranvier (ANK3) | .01 | −1.26 | .23 | −1.11 |
| Contactin 1 (CNTN1) | .30 | −1.09 | .76 | 1.03 |
| Contactin-associated protein-like 2 (CASPR2) | .19 | −1.23 | .12 | −1.25 |
| Neuronal cell adhesion molecule (NRCAM) | .01 | −1.30 | .51 | 1.09 |
| Tenascin-R (TNR) | .24 | −1.19 | .56 | 1.13 |
| Gliomedin (GLDN) | .23 | −1.23 | .77 | −1.06 |
| Sodium channel, voltage-gated, type II, α subunit, Nav1.2 (SCN2A) | .70 | −1.03 | .79 | −1.02 |
| Sodium channel, voltage-gated, type VIII, α subunit, Nav1.6 (SCN8A) | .04 | −1.34 | .16 | −1.24 |

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

a 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).
b Differently changed genes (P < .05).
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 BD$^{10-13}$ and SZ$^{14}$ 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 (permutated $P=.03$; 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 demographically homogeneous, healthy, young, white individuals without psychopathology (LOGOS cohort$^{27}$).

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 BD$^{12,18,19}$ consistent with these results.
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.8,30 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.35-36 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.35-36 That many of these proteins demonstrated to be crucial to the normal functioning of the NOR and to saltatory 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.35-36 One speculative mechanism is that NOR abnormalities will affect the conduction velocity, leading to attenuated, slow, abnormally variable or absent action potentials.3 Indeed, in an ANK3 knockout mouse model, the generation and persistence of action potentials were dramatically inhibited.37 Such abnormalities will limit the distance over which cortical neurons can fire in synchrony, affecting the normal long-range γ-oscillations between cortical subfields that could lead to attention deficits.4

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,38 including the Nav channels that are required for action potential generation.37 Cruz et al39 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 AnG 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

### Table 3. Genetic Association Analysis of ANK3 rs9804190 Polymorphism in the Learning on Genetics of Schizophrenia Spectrum Cohort4

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>UNPHASEDb</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Task</td>
<td>Outcome Variable</td>
<td>P Value</td>
</tr>
<tr>
<td>N-back</td>
<td>2-Back correct</td>
<td>.003</td>
</tr>
<tr>
<td>WCST</td>
<td>3-Back correct</td>
<td>&lt;.001c</td>
</tr>
<tr>
<td>Categories</td>
<td></td>
<td>.008c</td>
</tr>
<tr>
<td>Total errors</td>
<td></td>
<td>.008c</td>
</tr>
<tr>
<td>NTE</td>
<td>.009c</td>
<td></td>
</tr>
<tr>
<td>NPE</td>
<td>.02</td>
<td></td>
</tr>
<tr>
<td>TCI</td>
<td>Self-directedness</td>
<td>.01c</td>
</tr>
<tr>
<td></td>
<td>Self-transcendence</td>
<td>.01c</td>
</tr>
</tbody>
</table>

Abbreviations: NPE, Nelson nonperseverative; NTE, Nelson-type perseverative; TCI, Temperament and Character Inventory; WCST, Wisconsin Card Sorting Test.

The mean (SD), effect size, and adjusted P values are from a permutation test for association of outcome variables with those that reached significance at P < .05. The add value represents the estimated additive genetic value for the minor allele relative to the more common allele. A negative value signifies a lower score in the risk allele.

In the following outcome variables included in the UNPHASED analysis: for acoustic startle and prepulse inhibition: baseline startle (in 115-dB pulse-alone trials) and prepulse inhibition in 6 prepulse-pulse trial types (75 dB, 30 milliseconds; 75 dB, 60 milliseconds; 75 dB, 120 milliseconds; 85 dB, 30 milliseconds; 85 dB, 60 milliseconds; 85 dB, 120 milliseconds); for neurocognitive assessment: spatial working memory (between errors, 8-box condition; within errors, 8-box condition; stockpots of Cambridge (problems solved correctly); rapid visual information processing (A, B); N-back (1-, 2-, and 3-back correct); Stroop Interference Task (interference); WCST score (categories achieved, total errors, cards, NTE, NPE); word list (immediate, short delay, and long delay correct responses); and for personality questionnaires: State-Trait Anxiety Inventory, behavioral inhibition and activation system (reward responsive, drive, fun seeking), Eysenck Personality Questionnaire (psychoticism, extraversion, neuroticism), TCI (novelty seeking, harm avoidance, reward dependence, persistence, self-directedness, cooperativeness, self-transcendence), schizotypy questionnaire (magical thinking, paranoid ideation, unusual experiences).

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of shared genetic vulnerabilities between BD and SZ.\textsuperscript{12,18,19}

can 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, indica-
tions 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.

Figure 2. Regional brain activation during 3-back vs 0-back contrast.

A, Homozygotes of the ANK3 rs9804190 risk allele (CC; \(n=31\)) show significantly increased activation compared with carriers of the T allele (CT/TT; \(n=21\)) in 2 clusters within the inferior frontal gyrus (IFG) and middle frontal gyrus (MFG) (whole-brain analysis, \(P<.05\) with false discovery rate correction). B, Effect size of the mean activation levels of the respective suprathreshold clusters.

strategy of a deductive, direct, postmortem neurobiology
study minimized serendipitous findings, it did
involve multiple statistical tests. Thus, the potential for spu-
rarious associations because of multiple testing is an
important consideration. However, a consistent pattern
of ANK3 rs9804190 association was found in healthy sub-
jects, involving cognition and functional imaging, and
in ANK3 gene and protein expression levels in brain tis-
sue. 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 BY\textsuperscript{10,11} and SZ,\textsuperscript{14} further supporting the notion of
shared genetic vulnerabilities between BD and SZ.\textsuperscript{12,18,19}

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 hy-
pothesis-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 aug-
ment the effect size of ANK3 genetic variants. We iden-
tified an effect of the rs9804190 C allele in a larger
population of healthy subjects (LOGOS cohort) and using
a sensitive approach (neuroimaging cohort), further sup-
porting the notion of inadequate power.

Antipsychotic medication exposure is a confounding
factor in postmortem studies of SZ and therefore can con-
tribute to the detected gene expression abnormalities of
the axoglial genes. We did not find an altered expres-
sion level in the cerebral cortex of rats treated with halo-
peridol. However, the possibility of antipsychotic medi-
cation exposure confounds cannot be entirely ruled out,
as haloperidol exposure in rats might not exactly mimic the
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