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Convergent Findings for Abnormalities of the NF-κB Signaling Pathway in Schizophrenia

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Neurons exhibit a constitutive level of nuclear factor-κB (NF-κB) signaling and this pathway plays a significant role in neurite outgrowth, activity-dependent plasticity, and cognitive function. Transcription factor analysis was performed in a microarray data set profiled in four different brain regions ($n = 54$ comparison group; $n = 53$ schizophrenia (SZ)). An independent postmortem cohort was used for gene expression ($n = 24$ comparison group; $n = 22$ SZ), protein abundance ($n = 8$ comparison group; $n = 8$ SZ), and NF-κB nuclear activity ($n = 10$ comparison group; $n = 10$ SZ) quantification. Expression quantitative trait locus analysis was performed using publicly available data. Prepulse inhibition (PPI) of the acoustic startle reflex was tested in healthy individuals ($n = 690$). Comparison of microarray data showed that NF-κB was among the transcription factors associated with the differential expression of genes in cases *vs* controls. NF-κB gene and protein levels and nuclear activation were significantly decreased in the superior temporal gyrus of patients with SZ. Upstream NF-κB genes related to translocation were significantly dysregulated in SZ. The gene expression levels of an NF-κB-associated importin (KPNA4: one of the proteins responsible for the translocation of NF-κB to the nucleus) was decreased in SZ and an SNP within the KPNA4 locus was associated with susceptibility to SZ, reduced KPNA4 expression levels and attenuated PPI of the startle reflex in healthy control subjects. These findings implicate abnormalities of the NF-κB signaling pathway in SZ and provide evidence for an additional possible mechanism affecting the translocation of NF-κB signaling to the nucleus.
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INTRODUCTION

The nuclear factor-κB (NF-κB) signaling pathway has been recognized as an important regulator of the growth and morphology of neural processes in the developing and mature nervous system (Gutierrez and Davies, 2011). Diverse extracellular signals activate NF-κB, including inflammatory cytokines, such as TNF-α, neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and nerve growth factor, the excitatory neurotransmitter glutamate, and cell adhesion molecules (Gutierrez and Davies, 2011). NF-κB is constitutively activated in neurons, regulating the expression of an increasingly recognized number of genes involved in cell survival, synaptic plasticity, and memory in the adult brain (Gutierrez and Davies, 2011).

The NF-κB family of transcription factors consists of five members: p65 or *RELA*, p50, p52, c-REL, and RELB, which interact with each other by forming heterodimers or homodimers. In resting cells, NF-κB dimmers are normally kept in an inactive state by association with proteins of the NF-κB inhibitor family (IκB) (Perkins, 2007). In neurons, the most prominent heterodimer is the p65 and p50 complex, which is inactive in the cytoplasm when bound to IκBα (Gutierrez and Davies, 2011). However, other complexes are present in neurons and their subunit composition may vary depending on factors such as the developmental state and the location of neurons within the nervous system (Gutierrez and Davies, 2011). Activation of the canonical NF-κB pathway is controlled by the IκB kinase (IKK) complex. Following cell stimulation, the IKK complex phosphorylates IκB proteins on specific serine residues and targets IκB to polyubiquitination and proteasomal degradation liberating the NF-κB complex. The NF-κB dimmers translocate to the nucleus through interactions with importins, such as α3 and 4 (Fagerlund *et al*, 2005), and activate gene transcription by binding to κB sites in promoters of target genes.

There is growing evidence supporting a role for NF-κB in schizophrenia (SZ). First, NF-κB is activated by cytokines, neurotrophic factors, such as BDNF and glutamate, which have been strongly associated with SZ (for review see

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Buckley *et al*, 2011; Dean, 2011; Javitt *et al*, 2011; Watanabe *et al*, 2010). Second, first-episode, drug naive, schizophrenic patients show higher levels of cytokines in the serum and NF- κB activation (increased *RELA* gene expression and p65 nuclear activity) in peripheral blood mononuclear cells (Song *et al*, 2009). Third, pathway analysis using SZ candidate genes identified NF-κB as a hub, where multiple, diverse signal transduction pathways, enriched for SZ genetic risk factors, converge (Sun *et al*, 2010). Fourth, a recent study reported a genetic association for the *RELA* gene and *in silico* analysis suggested that the SZ risk genetic variants were associated with downregulation of *RELA* (Hashimoto *et al*, 2011).

The current studies were undertaken to advance the observation of NF-κB gene and protein expression abnormalities in SZ and explore further mechanisms of NF-κB regulation of transcriptional machinery. Exploratory analysis of data from a large postmortem microarray study of multiple brain regions of persons with SZ suggested downregulation of the NF-κB complex, as well as, alterations in the expression levels of upstream genes important for the activation and translocation of the NF-κB. The involvement of the NF-κB genes and proteins in SZ was more directly identified by analysis of postmortem brain tissue from an independent cohort of persons with SZ and unaffected comparison controls. Molecular (gene expression), cognitive (prepulse inhibition (PPI) of the acoustic startle reflex), and clinical data were used to examine whether genetic variants influence the NF-κB. *In silico* and genetic association studies identified a genetic variant in an importin gene that is associated with increased risk for SZ, reduced expression of the importin gene, and attenuated PPI of the acoustic startle reflex in human subjects.

MATERIALS AND METHODS

Postmortem Cohort

Brain tissue specimens were derived from the Brain Bank of the Department of Psychiatry of the Mount Sinai School of Medicine (New York, NY)/JJ Peters VA Medical Center (Bronx, NY). Total RNA was extracted from 50 mg of frozen tissue and gene expression was determined using the Affymetrix Human Genome U133 Plus 2.0 arrays (Santa Clara, CA) or quantitative polymerase chain reaction (qPCR). For the microarray analysis gray matter from dorsolateral prefrontal cortex (Brodmann area (BA) 46), middle temporal area gyrus (BA21), temporopolar area (BA38), and anterior cingulate cortex (BA32) from 21 SZ cases and 19 controls was used, as described in detail previously (Roussos *et al*, 2012b). Gray matter from the superior temporal gyrus (STG) and primary visual cortex (PVC) were used in qPCR analysis in an independent set of brain tissue specimens (22 SZ cases and 24 controls), similarly to previous studies (Roussos *et al*, 2012a) (Supplementary File). TaqMan probe ID's are listed in Supplementary Table 1. Similarly prepared sister aliquots from STG were used in quantitative western blotting ($n = 8$ per group) and NF-κB activation assay ($n = 10$ per group). Supplementary Table 2 shows the characteristics of the different postmortem cohorts used in the current study.

Healthy Individuals

We examined the association of the importin α 3 gene (*KPNA4*) rs4130284 with PPI of the acoustic startle reflex in 690 individuals from the LOGOS (Learning On Genetics Of SZ Spectrum) cohort, which has been described in detail previously (Roussos *et al*, 2011a,b). The Caucasian ancestry of these subjects was confirmed based on EIGENSOFT analysis (Patterson *et al*, 2006; Price *et al*, 2006) of genome-wide genotyping SNP profiling with the Illumina HumanOmniExpress BeadChip (San Diego, CA).

Quantitative Western Blotting

Blots were probed with rabbit anti-p65 polyclonal antibody (1:500 v/v dilution; Abcam, Cambridge, MA) and mouse anti-GAPDH antibody (1:10000 v/v dilution, Meridian Life Science, Saco, ME). Visualization and quantification of bands were performed with the Odyssey 2.1 software (Li-COR Biosciences, Lincoln, NE). To account for gel to gel variability, the relative expression value of p65 and GADPH in each sample was calculated as a ratio between the averaged intensities of the band in the experimental sample and in the 'standard-calibrator' (a mix of small aliquots of tissue from all samples run in parallel lanes).

NF-κB Activation Assay

Activation of the p65 subunit of NF-κB was determined using an NF-κB chemiluminescent DNA-binding enzyme-linked immunosorbent assay (ELISA) kit (TransAM™ NFκB Chemi Kits; Active Motif, Carlsbad, CA) according to the manufacturer's protocol in nuclear extracts from STG. The NF-κB detecting antibody recognizes an epitope on p65 that is accessible only when NF-κB is activated (Renard *et al*, 2001).

Chronic Haloperidol Studies in Rats

To assess the effects of neuroleptic exposure on the expression of selected genes of interest, groups of eight male Sprague-Dawley rats (6–8 months of age) received daily subcutaneous injections of haloperidol (2 mg/kg) or saline vehicle for 21 days. *Rela* and *Nfkb1* expression was assessed by qPCR using rat-specific primers and probes (Supplementary Table 1).

Statistical Data Analysis

Transcription factor analysis was carried out using the Ingenuity Pathway Analysis (IPA) (www.ingenuity.com) (Redwood City, CA). This analysis is based on literature-derived evidence on the directional ('activated' or 'inhibited') downstream influence of transcription factors on the expression level of transcripts included in experimentally derived gene expression data set. If the direction of change in the expression of a gene is consistent with the literature across most targets, IPA predicts that the transcription factor is more active in the experimental sample than in the control. If the direction of change is mostly inconsistent with the literature, IPA predicts that the transcription factor is less active in the experimental sample

than in the control. This analysis approach predicts a *Z*-score based on transcriptional regulators that are able to explain observed gene expression changes and infer their activation state. The *Z*-score determines whether an upstream transcription regulator has significantly more 'activated' predictions than 'inhibited' predictions ($z>0$) or vice versa ($z<0$).

Expression quantitative trait loci (eQTL) analysis was conducted in the BrainCloud data set ($n=268$) (http://braincloud.jhmi.edu) (Colantuoni *et al*, 2011) using linear regression models for SNPs located within 1 Mb of the transcript being tested (*cis*-eQTL). The Psychiatric Genomics Consortium GWAS results in SZ (Ripke *et al*, 2011) were visualized using ricopili (http://www.broadinstitute.org/mpg/ricopili/). A two-tailed Student's *t*-test was used to compare relative mRNA expression of NF-κB in qPCR, relative abundance of p65 protein and p65 activation assay. For multiple testing corrections in the mRNA gene expression comparisons, adjusted *P*-values were calculated based on 1000 permutations. A genotype by prepulse by interval ANOVA was used for PPI comparison in the LOGOS cohort. Student's *t*-test and ANOVA were performed using SPSS (version 20; IBM, Armonk, NY).

RESULTS

Microarray Data Set Analysis Suggests Downregulation of the NF-κB Pathway in SZ

The IPA transcription factor analysis was used in order to identify the transcription factors that are responsible for gene expression changes in a microarray data set from a recently published study (Roussos *et al*, 2012b). Given the observed gene expression changes in the experimental data set, the NF-κB (complex) and *RELA* were predicted to be downregulated in SZ in all brain regions whether examined separately or in total (Table 1). The specificity of the analysis is indicated by the fact that across all brain regions, out of 320 significant transcription factors (overlap *P*-value <0.05), only 39 were predicted with specific directionality (12 activated and 27 inhibited). The NF-κB (complex) was the most significant (lowest *Z*-score) inhibited transcription regulator in this data set. Furthermore, the majority of the NF-κB signaling genes were downregulated in the microarray data set (Supplementary Figure 2).

Confirmation of Gene Expression Changes of NF-κB Complex in STG by qPCR

Genes that encode the most prominent proteins of NF-κB complex (*NFKB1*, *RELA*, and *NFKBIA*) or are involved in the NF-κB signaling pathway (*MAP3K7*) in the STG and PVC were selected for qPCR analysis in a cohort of cases and controls. In the STG, the *RELA* (adjusted $P=0.021$; FC $=-1.56$) and *MAP3K7* (adjusted $P=0.01$; FC $=-1.36$) genes were significantly downregulated in SZ (Table 2). Similar changes were not observed in the PVC. Sample pH, PMI, or age of the donors did not correlated significantly with gene expression levels. ANCOVAs analysis with age, sex, pH, RIN, and smoking status as covariates did not change the results (Table 2). Similarly, *Rela* and *Nfkb1* mRNA expression levels were not altered in rats treated with haloperidol for 3 weeks *vs* vehicle (*P*-values >0.7 in both comparisons).

Decreased p65 Protein Levels and Nuclear Activation in STG of Individuals with SZ

To further explore the decreased *RELA* expression levels in STG, the p65 protein levels ($n=8$ per group) and nuclear activation ($n=10$ per group) were measured in samples from individuals with SZ *vs* controls (Supplementary Table 1). Samples from patients with SZ had decreased p65 protein levels ($P=0.009$) (Figure 1a) and reduced p65 nuclear activation ($P=0.009$) (Figure 1b). ANCOVAs analysis with age, sex, pH, and smoking status as covariates did not change the results for p65 protein levels ($P=0.017$) or nuclear activation ($P=0.036$).

Upstream RELA Genes Are Significantly Altered in SZ

Using the IPA gene data sets, we generated a list of genes (Supplementary Table 3) that affect the activation, activity, binding, expression, phosphorylation, and translocation of *RELA* and examined whether these genes exhibited altered gene expression. Overall, a significant proportion of the *RELA* upstream genes (29 out of 117 total transcripts) showed significant ($P<0.05$) changes in the microarray data set (Supplementary Figure 1; Supplementary Table 3). Genes related to translocation (15 out of 35 total transcripts) and phosphorylation (11 out of 30 total transcripts) showed the most significant alterations in the microarray data set, while genes related to binding and activation were the least affected in the microarray data set (Supplementary Table 3).

The Importin α 3 Gene (KPNA4) is Significantly Downregulated in SZ

Given the significant changes in the upstream genes related to translocation and the important role of importins in the translocation of the p65 protein in and out of the nucleus

Table 1 The IPA Transcription Factor Analysis Identified the NF-κB (Complex) and RELA Transcription Factors as Inhibited Based on the Gene Expression Changes in the Microarray Data Set

Transcription Factor	Target molecules in data set	*P*-value overlap	Predicted activation state	*Z*-score (number of genes with predicted inhibition)				
				All regions	BA21	BA32	BA38	BA46
NF-κB (complex)	246	8.9×10^{-16}	Inhibited	−4.9 (119)	−3.5 (110)	−3.5 (109)	−5.0 (123)	−2.6 (113)
RELA	125	1.7×10^{-14}	Inhibited	−2.2 (48)	−1.7 (46)	−1.8 (48)	−1.9 (48)	−1.0 (47)

Table 2 qPCR Analysis of Genes Implicated in the NF-κB Signaling in the STG and PVC in Controls ($n = 24$) and Cases with SZ ($N = 22$)

Gene name (symbol)	STG		PVC	
	P-value	FC ratio[a]	*P*-value	FC ratio[a]
Nuclear factor of κ light polypeptide gene enhancer in B-cells 1 (*NFKB1*)	0.12 (0.11)	−1.19	0.056 (0.08)	1.3
V-rel reticuloendotheliosis viral oncogene homolog A (avian) (*RELA*)	**0.021** (**0.02**)	−**1.56**	0.08 (0.29)	1.42
Nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor, α (*NFKBIA*)	0.4 (0.33)	−1.12	0.4 (0.9)	1.12
Mitogen-activated protein kinase kinase kinase 7 (*MAP3K7*)	**0.01** (**0.03**)	−**1.36**	0.2 (0.25)	1.24

[a]FC ratio-fold change ratios represent the ratio of geometric means for each gene to the three housekeeping genes in SZ/control (+) and in control/SZ (−) values. Differentially changed genes ($P < 0.05$) are highlighted in bold. Adjusted *P*-values calculated based on 1000 permutations are shown. In parenthesis are the *P*-values calculated using ANCOVAs with pH, age, sex, and RIN as covariates.

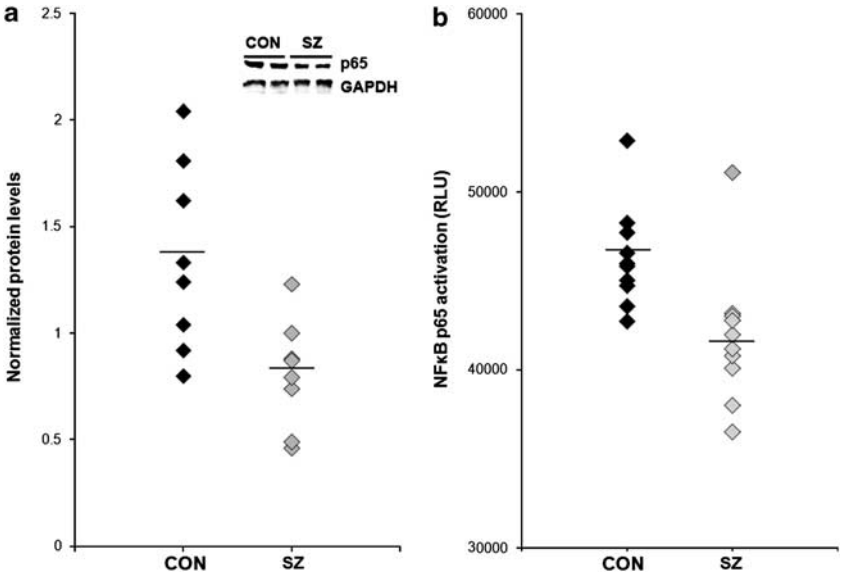

Figure 1 (a) Western blot quantification of p65 relative abundance in the STG. Individuals with SZ have decreased p65 levels compared with control subjects ($p = 0.009$). Data are expressed as means of p65 ODs normalized to GAPDH and to the amount of total protein ($n = 8$ per group). (b) NF-κB p65 nuclear activation in the STG. Individuals with SZ have decreased p65 nuclear activity compared with control subjects ($P = 0.009$) ($n = 10$ per group). RLU, relative light unit. Horizontal line displays the group means.

(Fagerlund *et al*, 2005), we examined in the qPCR cohort whether importin α 3 (karyopherin α 4 - *KPNA4*) and α 4 (karyopherin α 3 - *KPNA3)* gene expression is altered in SZ. In the STG, *KPNA4* (adjusted $P = 0.016$; FC $= -1.23$) was significantly downregulated, while a trend was observed for *KPNA3* (adjusted $P = 0.1$; FC $= -1.15$). No changes were found in the PVC (all adjusted *P*-values > 0.3).

In Silico Expression Quantitative Trait Locus Analysis Identifies KPNA4 as a Susceptible Gene

Using a hypothesis-driven approach, we screened for *RELA*, *KPNA3*, and *KPNA4* eQTLs using the BrainCloud data set (Colantuoni *et al*, 2011). Two *cis*-eQTLs (rs6793037 and rs4130284) were identified for *KPNA4* at $P < 10^{-3}$. Based on publicly available results from recent genome-wide association study (GWAS) in SZ (Ripke *et al*, 2011), rs4130284 was statistically associated with SZ ($P = 0.002$; odds ratio (OR) ± confidence interval (CI): 1.13 ± 0.04) while rs6793037 was not associated ($P = 0.36$; OR ± CI: 1.03 ± 0.03). rs4130284 demonstrated the same directional effect on gene expression and SZ susceptibility. More specifically, the rs4130284 T allele, that increases the risk for SZ, was associated with reduced *KPNA4* expression ($P = 0.0004$) (Supplementary Figure 3), as predicted based on the qPCR gene expression findings (*KPNA4* was significantly downregulated in SZ-adjusted $P = 0.016$).

The KPNA4 rs4130284 T Allele is Associated with Reduced PPI

There were no differences in demographic and startle variables between the three genotype groups (C/C, C/T, and T/T) (Supplementary Table 4). A mixed-model ANOVA of PPI with genotype as the grouping factor (three levels) and prepulse and interval as the within-subject factors revealed significant main effects of genotype [$F(2,687) = 4.32$, $P = 0.014$, $\eta^2 = 0.012$]. *Post hoc* comparisons with the Tukey's HSD (Honestly Significant Difference) test correction revealed that PPI of the C/C group was greater at a

trend level than PPI of the C/T group ($P = 0.1$) and the T/T group ($P = 0.075$). When carriers for the minor allele (T allele: C/T and T/T subjects) were grouped together, a significant main effects of genotype [$F(1,688) = 5.56$, $P = 0.019$, $\eta^2 = 0.008$] was observed in the mixed-model ANOVA, where carriers of the risk allele had reduced PPI compared with C/C subjects. Results were not altered when age, education, smoking status (cigarettes/day), and/or baseline startle were entered as covariates.

DISCUSSION

These results point to an abnormality in the expression of genes and proteins associated with the NF-κB signaling pathway in SZ. The gene expression results are strengthened by replication in two independent cohorts using diverse methods. Analysis of microarray data from four different brain regions showed that NF-κB signaling is overall inhibited and partially explains some of the downstream alterations in the gene expression found in elderly patients with SZ. The involvement of NF-κB signaling genes, protein, and nuclear activation assay in SZ was directly tested in postmortem brain tissue from an independent and larger sample of well-matched cases and controls. We found consistent lower expression of NF-κB-related genes and p65 protein, as well as decreased p65 nuclear activation, validating and extending the microarray findings. Furthermore, significant enrichment of altered genes based on the microarray analysis was observed for upstream regulators of the NF-κB signaling. Genes related to translocation showed the most prominent changes and based on these findings we further tested, in a hypothesis-driven approach, whether importins are altered in SZ. The gene expression of *KPNA4* was decreased in SZ and the T allele of the rs4130284 SNP was associated with attenuated *KPNA4* expression levels, disrupted PPI of the startle reflex and risk for SZ.

The NF-κB signaling pathway is ubiquitously expressed, but it has been studied most extensively in the immune system where it regulates a myriad of important physiological processes including immune and inflammatory responses, cell survival, and cell proliferation (Vallabhapurapu and Karin, 2009). However, growing evidence support an additional important role of NF-κB in the developing and mature nervous system (Gutierrez and Davies, 2011). More specifically, NF-κB has important role in controlling axon initiation (Sanchez-Ponce *et al*, 2008), elongation (Sanchez-Ponce *et al*, 2008), guidance (Mindorff *et al*, 2007), and branching (Gavalda *et al*, 2009) and in regulating dendritic arbor size and complexity during development (Chacon *et al*, 2010; Li *et al*, 2010; Salama-Cohen *et al*, 2005) and dendritic spine density in the adult (Christoffel *et al*, 2011; Russo *et al*, 2009). Furthermore, NF-κB is involved in other important functions including the regulation of neurogenesis in the embryo and adult (Denis-Donini *et al*, 2008; Koo *et al*, 2010; Young *et al*, 2006), cell survival in certain populations of peripheral and central neurons (Li *et al*, 2000) and promoting peripheral nerve myelination (Nickols *et al*, 2003). In the mature nervous system, a substantial body of evidence has implicated NF-κB as an important factor for fear conditioning, spatial memory, synaptic plasticity, and long-term memory (Dash *et al*, 2005; Freudenthal *et al*, 2005; Yeh *et al*, 2002). Given the above important functions that NF-κB subserves in the developing and adult brain, it is plausible that NF-κB signaling dysregulation can affect critical processes during neurodevelopment that increases susceptibility for SZ. This notion is further supported by the role of cytokines, neurotrophic factors, such as BDNF, and glutamate as activators of NF-κB signaling, which have all been associated with SZ (for review see Buckley *et al*, 2011; Dean, 2011; Javitt *et al*, 2011; Watanabe *et al*, 2010).

We found downregulation of *KPNA4* in the STG of persons with SZ, an important protein that mediates nuclear translocation of the *RELA* (Fagerlund *et al*, 2005). While a variety of stimuli activate NF-κB via several distinct pathways, *KPNA4*-mediated translocation of NF-κB is a convergent mechanism that is required as part of the NF-κB cascade. The nuclear translocation is a highly regulated process and a necessary mechanism to overcome the unique spatiotemporal challenges of neuronal cells. More specifically, long-range signaling from axon terminals to the nucleus is critical during long-lasting forms of synaptic plasticity (Perry and Fainzilber, 2009). In this study we provide evidence not only for *RELA* and *KPNA4* down regulation, but also for a genetic locus that might influence this process by affecting *KPNA4* abundance, leading to disrupted PPI, a robust intermediate phenotype for SZ (Braff, 2011; Roussos *et al*, 2008a,b, 2009, 2011a,b), and increased vulnerability for SZ. Thus, importin-mediated NF-κB transport in neurons provides an additional mechanism explaining the reduced p65 nuclei activity in patients with SZ. However, our genetic finding should be interpreted with caution for two reasons. First, in the original hypothesis-free GWAS (Ripke *et al*, 2011), the genetic association did not reach genome-wide level of significance. Second, the rs4130284 is located 336 kb downstream of the *KPNA4* locus. Nevertheless, our *a priori* hypothesis-driven postmortem brain-based genetic association study that eliminates the necessity for multiple testing correction, the convergent genetic association findings with the same allelic directionality among different cohorts and previous findings describing distant (>100 kb) *cis*-eQTL effects (Schadt *et al*, 2008), provide evidence for the robustness of our findings. However, future studies should replicate these findings.

To our knowledge this is the first study assessing the NF-κB signaling pathway in postmortem tissue of patients with SZ. A recent study found activation of NF-κB signaling in the peripheral blood of first-episode, drug naive, SZ patients (Song *et al*, 2009). The opposite results observed in our study might be secondary to NF-κB pathway assessment in different tissue and stage of illness. NF-κB signaling involves a very complex pathway and changes observed in peripheral blood might not reflect NF-κB activity in the central nervous system. Furthermore, Song *et al* included first-episode patients who were moderately to markedly ill, while our study included chronic patients with SZ. Multiple studies have shown that compared with first-episode, chronic patients demonstrate more prominent deficits in outcome variables related to cognition, brain volume, and connectivity (Chan *et al*, 2011; Ellison-Wright *et al*, 2008; Friedman *et al*, 2008; Salisbury *et al*, 2002). Although

speculative, NF-κB signaling might be activated in acute psychotic patients followed by reduction with sustained chronicity. This pathway might represent a compensatory mechanism which fails during the course of illness with reduction of expression levels, leading to deficits in synaptic plasticity. Chronic, phasic activation of the pathway during decompensation of illness might lead to resistance and overall downregulation in later stages. Another recent study reported a genetic association of *RELA* with SZ and PPI deficits in a Japanese population (Hashimoto *et al*, 2011). *In silico* eQTL analysis revealed that the SZ risk genetic variants affect *RELA* gene expression in immortalized B-lymphocytes. While these findings indicate that *RELA* will be downregulated in SZ, in this study we failed to identify a strong eQTL effect in human brain tissue. A shortcoming of the current study is a lack of a theoretical framework that incorporates alterations in the NF-κB signaling pathway and the core features of SZ. As the holes in our knowledge and the NF-κB signaling pathway become filled, the development of a data-driven theoretical framework will become more realistic.

Several factors may affect the quality of RNA and proteins, and DNA-binding activities of transcription factors in postmortem human tissues. Neuroleptic exposure is a confounding factor in postmortem studies of SZ and therefore can contribute to the detected gene expression abnormalities of the NF-κB signaling pathway. *In vivo*, haloperidol (1 mg/kg) treatment for 21 days did not modify the nigral expression of the p65 subunit while clozapine (1 mg/kg) reduced p65 expression as quantified by western blotting (Saldana *et al*, 2006). Lower dose of haloperidol (1 mg/kg), clozapine (5 and 10 mg/kg), or risperidone (5 mg/kg) did not alter NF-κB expression, while higher doses of haloperidol (2 and 5 mg/kg), increased p65 activation in nuclear lysates of the brains of rats treated for 21 days (Bishnoi *et al*, 2008a,b). Finally, haloperidol-treated rats (1 mg/kg) for 1 week increased p65 DNA-binding activity in hippocampus and caudate putamen (Post *et al*, 2002). In general, although results among different studies are inconsistent, there is an overall effect of antipsychotic for either increase or no alteration of the p65 activity in rats treated chronically with neuroleptics. Our findings in the cortices of rats are consistent with no changes of *Rela* and *Nfkb1* expression levels, further supporting the conclusion that the present findings are not secondary to chronic exposure to neuroleptics. However, the possibility of antipsychotic medication exposure confounds cannot be ruled out entirely, as haloperidol exposure of rats might not mimic the neuroleptic-induced changes in the brain of patients with SZ accurately. One additional limitation of our study is the small number of samples used for the p65 protein quantification and nuclear activation.

Collectively, these findings identify SZ-associated abnormalities in NF-κB genes and proteins. Adaptations in the NF-κB system may be essential for persistent neuroplastic changes underlying SZ and associated impairment of cognitive functions. While these adaptations may be induced through several mechanisms, we provide evidence for a possible genetic and neurobiological mechanism affecting the translocation of NF-κB signaling that might be among the keys to the pathophysiology of SZ and to the development of targeted therapeutic interventions.

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DISCLOSURE

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)