Molecular Psychiatry (2007) 12, 946–957

www.nature.com/mp

ORIGINAL ARTICLE

Neuregulin1-induced cell migration is impaired in schizophrenia: association with neuregulin1 and catechol-*o*-methyltransferase gene polymorphisms

Y Sei[1,3], R Ren-Patterson[1,3], Z Li[1], EM Tunbridge[2], MF Egan[1], BS Kolachana[1] and DR Weinberger[1]

[1]*Clinical Brain Disorder Branch, Genes, Cognition, and Psychosis Program, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA and* [2]*Department of Psychiatry, University of Oxford, Warneford Hospital, Oxford, UK*

Neuregulin1 (NRG1), a candidate susceptibility gene for schizophrenia, plays a critical role in neuronal migration and central nervous system development. However, its relation to schizophrenia pathogenesis is unknown. Here we show that B lymphoblasts migrate to NRG1 through the ErbB-signaling system as observed in neuronal cells. We assessed NRG1-induced cell migration in B lymphoblasts from patients with schizophrenia and found that NRG1-induced migration is significantly decreased compared with control individuals in two independent cohorts. This impaired migration is related at least in part to reduced AKT phosphorylation in the patients. Moreover, the magnitude of NRG1-induced migration is associated with polymorphisms of the *NRG1* and *catechol-o-methyltransferase* genes and with an epistatic interaction of these genes. This study demonstrates that the migratory response of schizophrenia-derived cells to NRG1 is impaired and is associated with genetic variations in more than one schizophrenia susceptibility gene, providing a novel insight into potential neurodevelopmental mechanisms of schizophrenia.
Molecular Psychiatry (2007) **12,** 946–957; doi:10.1038/sj.mp.4001994; published online 17 April 2007

Keywords: schizophrenia; neuregulin1; migration; ErbB lymphoblast; catechol-*o*-methyltransferase

Introduction

Schizophrenia is a complex psychiatric disease with a strong genetic predisposition. Earlier studies with genome-wide linkage scans revealed a positive linkage to schizophrenia on chromosome 8p.[1–8] Subsequent linkage and association studies in different ethnic populations implicated *neuregulin-1* (*NRG1*) on the 8p locus as a candidate susceptibility gene for schizophrenia.[9–11] Follow-up studies performed in postmortem human brain[12–14] and in genetically engineered animals[9,15,16] have provided further evidence that support a potential involvement of NRG1-mediated signaling in the neuropathogenesis of schizophrenia. However, the mechanisms by which NRG1 contributes to susceptibility for schizophrenia remains unknown.

Correspondence: Dr Y Sei, Blood Genomics Laboratory, Genes, Cognition, and Psychosis Program, National Institute of Mental Health, 10 Center Drive, Bldg 10, Rm 4S235, MSC1379, Bethesda, MD 20892-1385, USA.
E-mail: seiy@mail.nih.gov or
Dr DR Weinberger, Clinical Brain Disorder Branch, Genes, Cognition, and Psychosis Program, National Institute of Mental Health, 10 Center Drive, Bldg 10, Rm 4S235, MSC1379, Bethesda, MD 20892-1385, USA.
E-mail: daniel.weinberger@mail.nih.gov
[3]These authors contributed equally to this work.
Received 5 June 2006; revised 1 December 2006; accepted 4 February 2007; published online 17 April 2007

NRG1 functions primarily by interacting with ErbB tyrosine kinase receptors and plays critical roles in synaptic plasticity[17,18] and regulation of neurotransmitter receptors, such as the *N*-methyl-*d*-aspartate receptor[19,20] the type I γ-amino butyric acid (GABA) receptor,[21,22] and the $\alpha 7$ nicotinic acetylcholine receptor[23] in the central nervous system. Interestingly, all of these processes have been independently implicated in the neuropathogenesis of schizophrenia.[24,25] NRG1–ErbB signaling also appears to play an important role in the migration and differentiation of neuronal and glial precursors early in brain development.[26–29] Anton *et al.*[30–33] demonstrated that NRG1–ErbB signaling is critical for modulating the elongation of cortical radial glia fibers in a culture system and suggest that perturbation of NRG1–ErbB signaling may result in abnormal cortical development.[26] Moreover, NRG1 has also been implicated in myelination processes by inducing the migration and differentiation of oligodendrocytes.[30–33] Therefore, alterations in NRG1–ErbB signaling could lead to a variety of abnormal neurodevelopmental events that have been implicated in the underlying pathology of schizophrenia.[34]

In this study, we addressed in a cell-model system the possibility that NRG1-mediated neural migration is affected in schizophrenia. Since no technique is available to assess neural migration in living human

brain, we used cells of hematopoietic origin from patients and controls to test their ability to migrate in response to NRG1. There is increasing recognition that neuronal cells and immune cells share many cellular and molecular mechanisms for cell migration/motility.[35] Moreover, conceptual support for the commonality of these phenomena comes from a recent finding that animals deficient for stromal cell-derived factor 1, a chemokine that mediates migration of lymphocytes and cerebellar neurons, or its receptor CXCR4 exhibited both abnormal cerebellar development and impaired B lymphopoiesis.[36–37] Thus, if susceptible genes are commonly expressed and functional in these two systems, abnormalities caused by the genes in hematopoietic cells might reflect those in neurons.

In the present study, we used B lymphoblasts developed from normal subjects and from patients with schizophrenia to characterize the effect of NRG1/ErbB signaling on the migratory behavior of these cells and then tested whether NRG1-induced migration of B lymphoblasts was impaired in the patients. Our data suggest that the migratory response of schizophrenia-derived cells to NRG1 is impaired and is associated with an NRG1 risk polymorphism and also with another gene implicated in psychosis, *catechol-o-methyltransferase* (*COMT*) gene, and with an epistatic interaction of these genes, providing novel insights into neurodevelopmental mechanisms related to genetic risk for schizophrenia.[38]

Materials and methods

Subjects

Blood collection and transformation of lymphocytes were approved by the NIMH institutional review board, and all donors provided informed consent. The first set of B lymphoblasts used to characterize ErbB-signaling was derived from 16 normal controls (eight females, eight males; age 34.5 ± 9.1 years at the time of blood collection, mean $\pm$ s.d.) and 14 individuals with schizophrenia (eight females, six males; age 36.7 ± 12.6 years). These subjects were drawn from individuals participating in the Clinical Brain Disorders Branch 'Sibling Study' protocol, an ongoing investigation of neurobiological abnormalities related to genetic risk for schizophrenia. The details of subject recruitment and examination are described elsewhere.[39] Following the results from the first set of experiments, a second set of B lymphoblasts was obtained from a new group of 32 subjects to test NRG1-stimulated migration. Twenty were from normal controls (10 females, 10 males; age 29.7 ± 9.9 years) and 12 were from individuals with schizophrenia (five females, seven males; age 37.9 ± 11.4 years). Only Caucasian subjects of self-reported European ancestry were included to avoid genetic stratification and to reduce heterogeneity. In addition, because of our prior interest in studying the effects of the COMT Val[158]Met polymorphism on various phenotypes, we selected B lymphoblasts from Val or Met homozygous individuals for both case and control groups to increase statistical power for comparisons. The number of samples included in each assay varied based on the availability of cells at the time of testing.

B lymphoblast culture

B lymphocytes in the mononuclear cell preparation were transformed by infection with Epstein–Barr virus (EBV) and maintained as described previously.[40] The transformed B lymphoblasts were grown in RPMI-1640 medium (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) (Cambrex, Walkersville, MD, USA), L-glutamine (2 mmol/l), 100 μg/ml streptomycin and100 U/ml penicillin (Gibco) in an incubator (95% air/5% CO_2 at 37°C).

Reagents

NRG1α (296-HR) was the 65 amino-acid residue recombinant protein from the epidermal growth factor (EGF) domain of NRG1α (177–241 and purchased from R&D system Inc. (Minneapolis, MN, USA). Antibodies to ErbB2 (sc-284), ErbB3 (sc-7390) and ErbB4 (sc-283) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Another antibody to ErbB3 (AP7630a) was from ABGENT (San Diego, CA, USA). Antibodies to Erk (06-182), AKT (07-416) and phospho-AKT (05-669) were from Upstate (Charlottesville, VA, USA). The antibody to phospho-ERK (sc-7383) was from Santa Cruz. Fibronectin (F3667) and a monoclonal antibody to β-actin (A5441) were from Sigma (St Louis, MO, USA). Broad ErbB inhibitor PD158780, ErbB1 inhibitor AG1478, ErbB2 inhibitor AG825, AKT inhibitor III, PI3K inhibitor wortmannin, phospholipase C (PLC)γ inhibitor U73122, and MAP kinase inhibitors U0126 and PD98059 were purchased from Calbiochem (La Jolla, CA, USA). These inhibitors were dissolved in dimethylsulfoxide except that U73122 was dissolved in ethanol.

Reverse transcription-PCR

Total RNA was extracted using the SV Total RNA Isolation System (Promega, Madison, WI, USA) and reverse transcription performed to generate the first strand of cDNA using a cDNA synthesis kit (Promega). Synthesized cDNA was then amplified by polymerase chain reaction (PCR) using a primer set which selectively amplifies specific members of the ErbB family. The sequences of the primers for ErbB2, ErbB3, and ErbB4 have been published previously.[41] The amplification was performed with negative and positive controls using the optimal number of cycles to ensure that the amplification was completed within the exponential range. Thus, PCR amplifications for ErbB2, ErbB3, ErbB4 and β-actin were performed with 32, 40, 40 and 25 cycles, respectively. The sequences of primers for β-actin were 5′-dAAG AGAGGCATCCTCACCCT-3′ (sense) and 5′-dTGC TGATCCACATCTGCTGGA-3′ (antisense). The signal ratio of ErbB to β-actin was determined on the basis of

the ratio of the intensity of the PCR product compared with the corresponding β-actin band. The PCR products were imaged and the relative optical density of each band was measured and analyzed using NIH Image software.

Imaging of cell migration
Cells (10^6/ml) were plated onto a fibronectin (5 μg/ml)-coated culture dish (60 mm) and maintained in RPMI-1640 medium containing 10% FBS, L-glutamine (2 mmol/L), 100 μg/ml streptomycin and 100 U/ml penicillin in a 5% CO_2 incubator at 37°C overnight. After free-floating cells were carefully removed by rinsing the culture twice with warm serum-free RPMI, the culture dish was placed on the microscope for 10 min. A micro-tip (Femtotips, Eppendorf, Germany), which was filled with 100 μg/ml NRG1α in phosphate-buffered saline (PBS) to generate chemical gradients of the chemoattractant, was placed into the culture dish.[42] A plastic lid was then placed to prevent disturbance of the media from local airflow. Cells were observed on an inverted microscope TE200 (Nikon, Tokyo, Japan). Movement of cells was recorded for an hour using a DXM1200 Digital Camera (Nikon) controlled by ACT-1 software (Nikon).

Chemotaxis assay using transwell migration methods
The transwell chemotaxis assay was carried out using a InnoCyte chemotaxis chamber with an 8-μm pore size (Calbiochem) or a QCM chemotaxis chamber with a 5-μm pore size (Chemicon, Temecula, CA, USA), according to the manufacturer's instructions. Briefly, cells were suspended at 4×10^5 cells/ml in serum-free RPMI-1640. Then, 100–150 μl of the cell suspension (40 000–60 000 cells) was applied to the upper wells of the chemotaxis chamber. Serum-free RPMI-1640 with or without NRG1 (200 μl/well) was added to the lower wells. After 4–24 h in an incubator (95% air/5% CO_2 at 37°C), cells attached to the lower side of the membrane were detached by detachment solution provided in the kit, lysed with 0.1% Triton X-100 and measured using CyQUANT GR double-stranded DNA detecting reagent (Molecular Probes, Eugene, OR, USA). For the InnoCyte kit migration assay, migrated cells were measured following labeling cells with Calcein-AM. The results were expressed as a 'chemotaxis index' calculated by the following formula: chemotaxis index = migration in response to NRG1/migration in response to vehicle control (baseline count). All assays were carried out in triplicate.

Immunoblot analysis
Cells were disrupted in disposable Dounce homogenizers in buffer containing 250 mM Tris-HCl (pH 7.4), 100 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.5% Deoxycholic acid, 1 mM *p*-nitrophenylguanidinobenzoate, and protease inhibitor cocktail I and II (Sigma) and then incubated for 20 min at 4°C. Following centrifugation at 14 000 *g* for 15 min, the supernatants were collected. The protein samples (20–50 μg per lane) were separated using SDS-PAGE on 4–12 or 8–16% Tris–glycine gel (Invitrogen, Carlsbad, CA, USA). After separation, the proteins were transferred to polyvinylidene difluoride membrane (Millipore Corp, Bedford, MA, USA), and then probed with the primary antibodies (0.5–1 μg/ml). Horseradish peroxidase-conjugated anti-rabbit or mouse IgG antibody (Pierce, Rockford, IL, USA) was used to detect the primary antibodies. Chemiluminescence detection was performed using enhanced chemoluminescence (Amersham Biosciences, Buckinghamshire, UK) or Super pico signal (Pierce). The protein bands within the linear range of the standard curve were imaged and the relative optical density of each band was measured and analyzed using *NIH Image* software.

Immunofluorescent staining
Cells were plated onto poly-L-lysine-coated glass coverslips and maintained in RPMI-1640 medium containing 10% FBS, L-glutamine (2 mmol/l), 100 μg/ml streptomycin and 100 U/ml penicillin in a 5% CO_2 incubator at 37°C overnight before stimulation, fixing and staining. Cells were washed with 0.01 M PBS (pH 7.4) and fixed in methanol for 20 min at room temperature. Fixed cells were first blocked with 10% normal goat serum in 0.01 M PBS at 37ºC for 30 min. Cells were then incubated with rabbit polyclonal antibody specific to ErbB2 (sc-284, Santa Cruz) or mouse monoclonal antibody to ErbB3 (sc-7390), which were optimally diluted in PBS (0.5–1 g/ml) containing 1% bovine serum albumin and 0.025% Triton X-100, at 4°C overnight. After washing, cells were incubated with goat-anti-mouse IgG-fluorescein isothiocyanate (Chemicon) and goat-anti-rabbit IgG-Tex-Red (Santa Cruz) for 2 h at 37°C Immunostaining was analyzed with the LSM 510 confocal laser-scanning microscope (Zeiss, Oberkochen, Germany).

Cell proliferation assay
The proliferation of B lymphoblasts (2×10^4 per well in 96-well plate) was measured by a colorimetric assay using WST-1 (Roche Applied Science, Indianapolis, IN, USA). The data were expressed as the stimulation index, which was calculated as the ratio of the experimental count to the spontaneous count.

Genotype determination
Four single nucleotide polymorphisms (SNPs) (common 1 allele > rare 2) in *NRG1* from the original Icelandic risk haplotype,[10] SNP8NRG221132 (G > A), SNP8NRG221533 (T > C), SNP8NRG241930 (G > T) and SNP8NRG243177 (C > T, rs6994992) and a single SNP in *COMT* (Val108/158Met, G > A, rs4680) were determined by 5′ exonuclease allelic discrimination TaqMan assay using probes and primers available from ABI as part of their 'Assays on Demand' program.

Statistical analysis
Between-group comparisons were performed by using unpaired *t*-tests or Mann–Whitney rank-sum test,

where applicable. The effect of genotypes on chemotaxis index was assessed by analysis of variance (ANOVA) and followed by Fisher's *post hoc* comparison matrices. The parametric relationship between the number of target allele and chemotaxis index was assessed using regression analysis. Data were expressed as mean ± s.e.m.

Results

Expression of ErbB2 and ErbB3 receptors in B lymphoblasts

We examined mRNA expression of receptors for ErbB2, ErbB3 and ErbB4, in B lymphoblasts using semi-quantitative reverse transcription (RT)-PCR (Figure 1a). Relative levels of ErbB2–4 expression were determined by comparison with levels of a 'housekeeping' gene, β-actin, and expressed as the signal ratio of ErbB to β-actin. ErbB2 and ErbB3 transcripts were expressed in all the B lymphoblasts tested in both controls and schizophrenia patients. No significant differences were seen between groups in levels of normalized ErbB2 (ErbB2/β-actin, control ($n=14$) vs schizophrenia ($n=12$); 0.63 ± 0.05 vs 0.67 ± 0.04, $P=0.50$, unpaired t-test) and ErbB3 (ErbB3/β-actin ratio, control ($n=14$) vs schizophrenia ($n=12$); 0.68 ± 0.05 vs 0.71 ± 0.03, $P=0.54$). We also verified the expression of ErbB2 ($\sim$185 kDa) and ErbB3 ($\sim$150 kDa) proteins by Western blotting (Figure1b and Supplementary Figure S1). ErbB4 mRNA expression was undetectable in some individuals by these methods (Figure 1a). No significant differences were found between clinical groups (control, $n=14$ vs schizophrenia, $n=12$) in the frequency of ErbB4-positive samples (16.7 and 14.3%, respectively, $P=0.867$, χ^2) or ErbB4/β-actin ratio ($P=0.79$, t-test). Detection of ErbB4 by Western blotting with our antibody has not been successful.

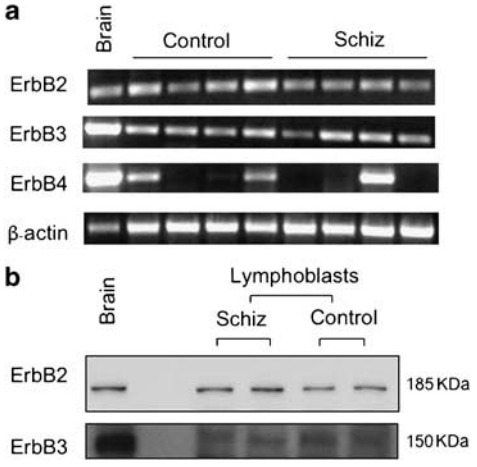

Figure 1 (**a**) Agarose gel electrophoresis of PCR products following selective reverse transcription-PCR for ErbB2, ErbB3, ErbB4 and β-actin. PCR products from B lymphoblasts of four patients and four controls are shown. The subjects were selected randomly with respect to genotype. The amplicons from human brain tissue in the first left lane serve as positive controls for these amplifications. Results shown are representative of at least three experiments. (**b**) Protein expression of ErbB2 and ErbB3 in human brains and B lymphoblast cell lines. Protein lysates from brain tissues (20 μg) and B lymphoblasts (50 μg) were subjected to Western blot analysis for ErbB2 and ErbB3 detection using anti-ErbB2 (sc-284, Santa Cruz) and anti-ErbB3 (AP7630a, ABGENT) respectively. The immunoblot showed expression of ErbB2 ($\sim$185 kDa) and ErbB3 ($\sim$150 kDa) in human brains (positive controls) and B lymphoblasts from two patients with schizophrenia and two control individuals. The subjects were selected randomly with respect to genotype. Results shown are representative of more than five experiments.

NRG1-induced colocalization of ErbB2 and ErbB3 receptors at the front-leading edge of migrating cells

To examine the cellular localization of ErbB2 and ErbB3 receptors, we performed an immunohistochemical analysis of the receptors using confocal microscopy (Figure 2a–d, Supplementary Figures S2 and S3). Immunostaining showed that ErbB2 and ErbB3 receptors are highly localized to the plasma membrane and diffusely in the cytoplasm of B lymphoblasts (see enlarged images in Supplementary Figure S3). We found no noticeable differences in the localization patterns between controls and patients with schizophrenia. NRG1 treatment induced cell polarization, which was accompanied by a striking redistribution of ErbB2 and relatively modest redistribution of ErbB3 receptors. In the cells exposed to NRG1, the ErbB receptors were clustered as a patch at the leading edge of the cells towards the highest concentration of NRG1 (Figure 2c and d). The NRG1-stimulated redistribution was more prominent in ErbB2 than in ErbB3. Both controls and patients showed visibly obvious NRG1-induced colocalization of ErbB2 and ErB3.

NRG1 stimulated-activation of Ras–MAPK and PI3K–AKT signaling pathways

NRG1 activates Ras–MAPK and phosphatidylinositol 3 kinase–protein kinase B (PI3K–PKB/AKT) pathways through ErbB receptors.[43] To determine whether NRG1 activates these pathways in B lymphoblasts, we measured phosphorylation of ERK at Tyr^{180} and AKT at Ser^{473}, which serve as an indication of Ras–MAPK and PI3K–AKT activation, respectively. Representative Western blots (top) and line graphs of phosphorylated form/total ratio (middle panel) demonstrate NRG1-stimulated phosphorylation of Erk (Figure 3a) and AKT1 (Figure 3b) in controls and schizophrenics. Our results showed that NRG1α increased the relative phosphorylation of ERK at Tyr^{180} and AKT at Ser^{473} in B lymphoblasts ($P=0.01$ for all and $P=0.04$ for controls, respectively, paired t-test), suggesting that NRG1α activated the Ras–MAPK and PI3K–AKT pathways (Figure 3a and b). There were no differences in peak fold increases in the NRG1-induced phosphorylation of ERK at Tyr^{180} between individuals with schizophrenia ($n=6$,

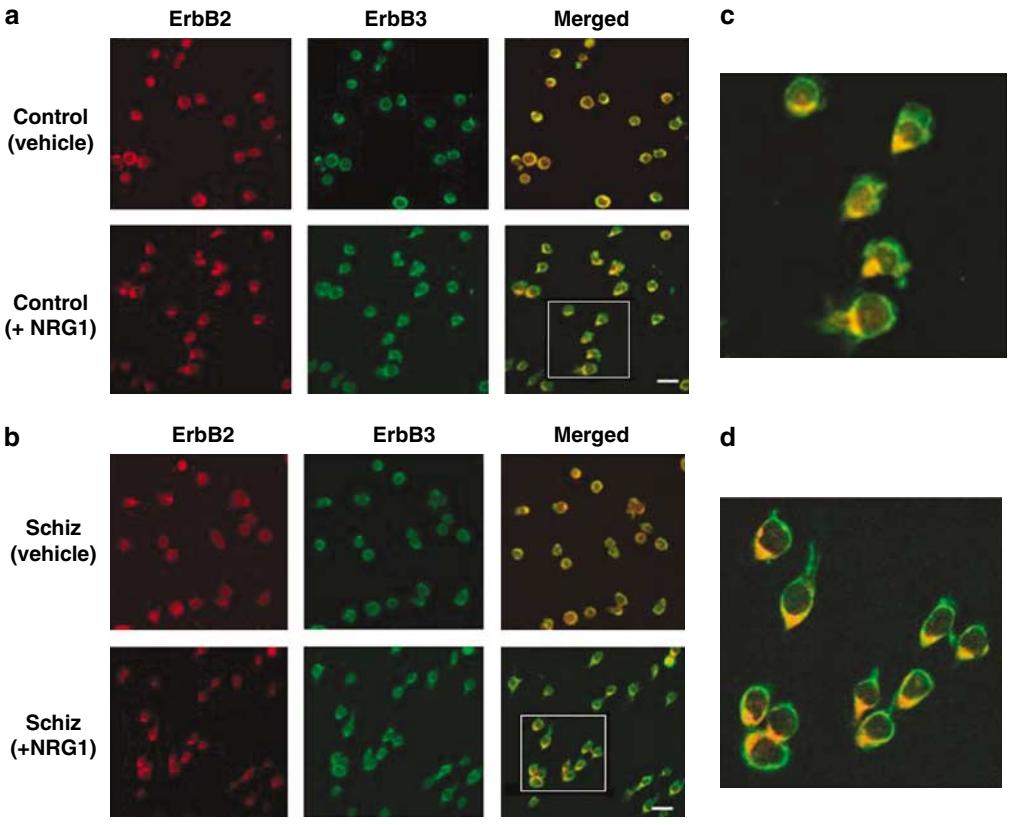

Figure 2 (**a**, **b**) Localization of ErbB2 and ErbB3 in B lymphoblasts from a control individual (**a**) and a schizophrenic patient (**b**). Untreated (upper panel) and NRG1-treated cells (lower panel) were immunostained for ErbB2 (red, left panel) and ErbB3 (green, middle) and observed by confocal microscopy as detailed in Materials and methods. The merged pictures are shown on the right panel. B lymphoblasts were stimulated with NRG1 and fixed 30 min after the stimulation. One microliter of NRG1 (50 μg/ml) or PBS (vehicle) was applied at the lower left corner of each chamber slide containing 0.5 ml culture media. The cells were fixed, permeabilized, and stained with anti-ErbB2 antibody (sc-284; Santa Cruz, 1:200 dilution), followed by Tex-Red-conjugated secondary antibody (red), or anti-ErbB3 antibody (sc-7390; Santa Cruz, 1:200 dilution), followed by fluorescein isothiocyanate-conjugated secondary antibody (green). Control staining without primary antibody showed no reactivity (data not shown). Scale bar = 40 μm. (**c**) An enlarged image of co-localized ErbB2 and ErbB3 from the lower right panel in (**a**) (squared region). Enlarged images of all 12 images are presented in Supplementary Figure S3. (**d**) An enlarged image of co-localized ErbB2 and ErbB3 from the lower right panel in (**b**) (squared region). Results shown are representative of more than five experiments.

2.04 $\pm$ 0.50-fold) and controls ($n = 6$, 1.46 $\pm$ 0.12-fold) ($P = 0.28$, unpaired *t*-test). In contrast, the NRG1-induced change in the relative phosphorylation of AKT at Ser^{473} was significantly lower in patients with schizophrenia ($n = 5$, 0.93 $\pm$ 0.06-fold) than in controls ($n = 6$, 1.30 $\pm$ 0.14-fold) ($P = 0.049$, unpaired *t*-test).

NRG1-induced cell migration of B lymphoblasts and its dependence on ErbB tyrosine kinase, PI3K–AKT and PLCγ

Because NRG1 activated the Ras–MAPK, PI3K–AKT (Figure 3) and PLCγ–Ca^{2+} signaling pathways (Supplementary Figure S4), we determined whether NRG1 could activate migration using B lymphoblasts from normal subjects. By imaging cells under the microscope, we observed that cell migration toward a microinjector containing a high concentration of NRG1α was accompanied by consistent changes in cell shape (Figure 4a). To quantify the migration of cells and to determine the critical signaling pathways required for this migration, we measured the degree of chemotactic response of B lymphoblasts using the transwell chemotaxis method. The transwell assay showed significant chemotactic responses of B lymphoblasts to NRG1α (200 ng/ml) ($P = 0.015$, *t*-test) (Figure 4b). We next determined whether NRG1-induced chemotaxis is mediated by ErbB tyrosine kinases. A selective ErbB2 inhibitor AG825 (1 μM) and a relatively broad ErbB inhibitor PD158780 (10 μM) both significantly inhibited NRG1-mediated chemotaxis ($P = 0.025$ and $P = 0.01$, respectively), whereas the selective ErbB1 inhibitor AG1478 (10 μM) failed to block this cell function ($P = 0.104$). We then tested whether Ras–MAPK, PI3K–AKT and PLCγ–Ca^{2+} signaling pathways were involved in the NRG1-mediated cell migration. The AKT inhibitor III (10 μM), PI3K inhibitor wortmannin (50 nM) and the PLCγ inhibitor U73122 (10 μM) significantly suppressed NRG1-induced chemotaxis ($P < 0.001$, Figure 4). MAPK inhibitors U0126 (100 nM) or

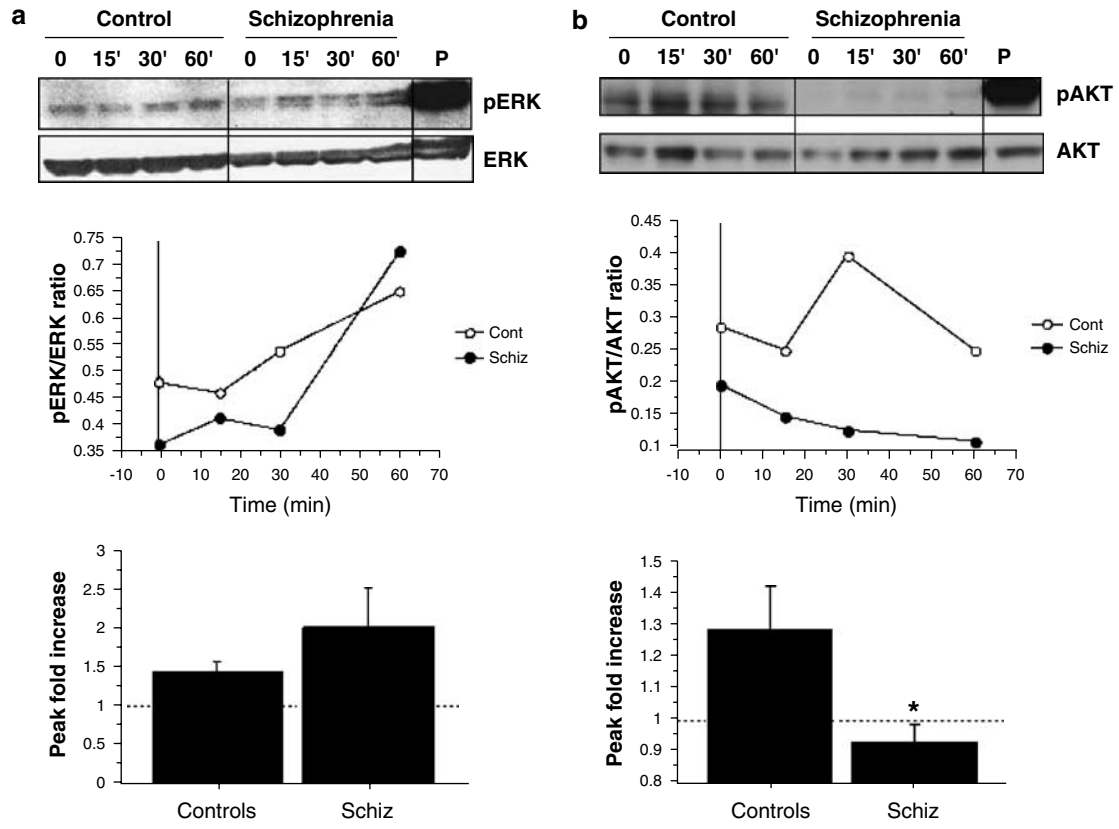

Figure 3 NRG1-stimulated phosphorylation of ERK and AKT1. B lymphoblasts from the first cohort (see Materials and methods) of control subjects and schizophrenic patients were stimulated with NRG1α (200 ng/ml) for the time points indicated (upper panels). Protein isolated from the cells was analyzed by Western blotting. Positive controls (P) were obtained from NRG1-stimulated PC12 cells. To quantify the level of phosphorylation, the immunoblots were stained with antibodies specific to phosphorylated ERK at Tyr^{180} (42, 44 kDa) (**a**) or AKT at Ser^{473} (60 kDa) (**b**) and were then stripped and reprobed with antibodies to total ERK or AKT, respectively. The lined graphs (middle panels) represent changes in the ratio of phosphorylated form to total ERK (or AKT) over time from the above immunoblots. The bar graphs represent peak fold increase of ERK and AKT1 over baseline (means ± s.e.m.) obtained from six controls and 5–6 patients with schizophrenia as detailed in Results (lower panels). $^*P = 0.049$, patients vs controls. The subjects were selected randomly with respect to genotype. The phosphorylated form/total ratio was calculated after a quantitative densitometric analysis of each band within a linear range and adjusted based on the ratio obtained from positive controls (P). The adjusted ratio was obtained for every sample for all the experiments and used for inter-experimental comparisons.

PD98059 (10 μM) had no significant effect on chemotaxis ($P = 0.086$ and $P = 0.65$, respectively).

Impaired NRG1-induced cell migration in patients with schizophrenia

To examine NRG1-induced cell migration in patients with schizophrenia, we tested cells using the transwell method in two conditions: 4 h chemotaxis through an 8-μm pore (Figure 5a) and followed by 24 h chemotaxis through a 5-μm pore to verify results from an 8-μm pore assay (Supplementary Figure 5). In the 4-h assay, NRG1α (200 ng/ml)-induced cell migration was significantly lower in the patients ($n = 13$, chemotaxis index 0.997 ± 0.020) than in controls ($n = 13$, 1.072 ± 0.13) ($P = 0.007$, Mann–Whitney test) (Figure 5a). Background migration in response to vehicle control was not significantly different between the two groups and NRG1α did not induce cell proliferation in either group (controls, $n = 14$, stimulation index 0.961 ± 0.018; schizophrenia, $n = 13$, stimulation index 0.987 ± 0.014, $P > 0.1$).

We obtained another group of patients with schizophrenia ($n = 12$) and another group of controls ($n = 20$) and performed a 4-h, 8-μm pore assay to confirm our previous finding of impaired NRG1-induced cell migration in schizophrenia (Figure 5b). In this new cohort, as seen in the previous groups tested, NRG1α (200 ng/ml)-induced cell migration was significantly lower in patients with schizophrenia ($n = 12$, chemotaxis index 0.994 ± 0.004) than in controls ($n = 20$, 1.041 ± 0.011) ($P = 0.0006$, Mann–Whitney test) (Figure 5b). Background nonspecific migration was comparable between the two groups.

Effects of NRG1 and COMT polymorphism on NRG1-induced cell migration

We tested whether variation in the NRG1 migration response across subjects might relate to genetic

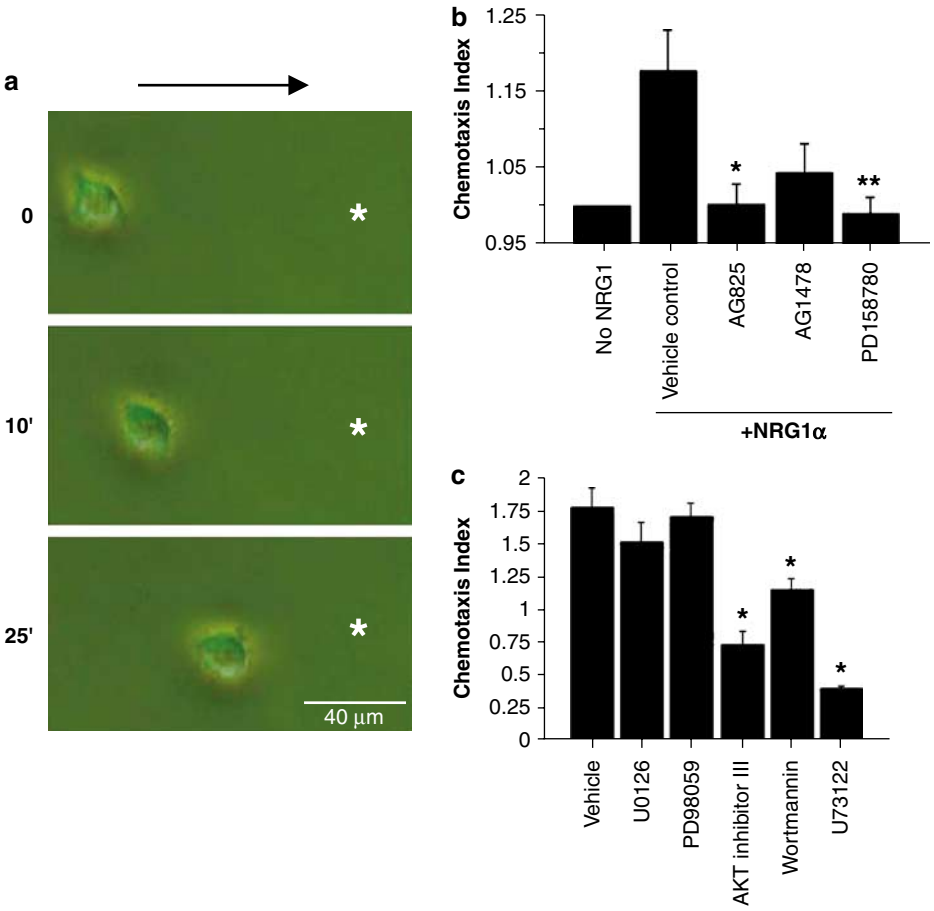

Figure 4 NRG1-induced cell migration of B lymphoblasts. (**a**) Images of B lymphoblast migrating in response to NRG1α at the indicated time points after NRG1α exposure. The arrow shows the direction of the position of the micro-tip. The asterisk indicates a landmark position. (**b**) Inhibition of NRG1α-induced cell migration by ErbB2 kinase inhibitors. B lymphoblasts from three control individuals were plated in the upper chamber of a Transwell filter with 5 μm pores and allowed to migrate for 24 h in the presence or absence of NRG1α (200 ng/ml). Cells subjected to NRG1-induced migration were treated with either dimethylsulfoxide (vehicle control), the ErbB2 inhibitor AG825 (1 μM), the ErbB1 inhibitor AG1478 (10 μM), or the ErbB2 inhibitor PD158780 (10 μM). Each treatment was repeated in triplicate. The inhibitors were added in the upper chamber. Data represent mean $\pm$ s.e.m. of chemotaxis index from three individuals. The control subjects were selected randomly with respect to genotype. *$P = 0.025$, **$P = 0.01$ (compared with cells treated with vehicle control). (**c**) Inhibition of NRG1α-induced cell migration by various kinase inhibitors. The assay conditions were the same as in (**b**). Cells were treated with dimethylsulfoxide (vehicle control), U0126 (100 nM), PD98059 (10 μM), the AKT inhibitor III (10 μM), wortmannin (50 nM) or U73122 (10 μM). All inhibitors were added in the upper chamber. Data represent mean $\pm$ s.e.m. of chemotaxis index from three individuals. The subjects were selected randomly with respect to genotype. *$P < 0.001$ (compared with cells treated with vehicle controls).

variation implicated in schizophrenia and in NRG1 function. As a preliminary investigation, we focused on two polymorphisms (1) an SNP (SNP8NRG243177) in the original Icelandic risk haplotype[10] associated with schizophrenia that has been shown to predict expression of a specific isoform of NRG1[44] and (2) a functional SNP in COMT (Val[158]Met), which is implicated as a risk factor in psychosis[24] and in metastatic cancer.[45] A two-way ANOVA (with NRG1 genotype and diagnosis as factors) revealed significant main effects of SNP8NRG243177 genotype ($F = 3.842$, df = 2,50, $P = 0.0281$) and of diagnosis ($F = 15.575$, df = 1,50, $P = 0.0002$) on NRG1-induced chemotaxis (Figure 6a). There was no significant interaction. *Post-hoc* testing revealed significantly reduced migration in genotypes containing risk alleles (T) (i.e., contrasting T/T and C/C genotypes ($P = 0.0006$) or C/C and C/T genotypes ($P = 0.02$)) in the entire sample. Another two-way ANOVA (COMT genotype and diagnosis) also revealed significant main effects of COMT genotype ($F = 6.806$, df = 1,54, $P = 0.0117$) and diagnosis ($F = 22.361$, df = 1,54, $P < 0.0001$) on NRG1-induced chemotaxis (Figure 6b) and that there was no interaction. *Post hoc* comparison revealed that in the group of patients with schizophrenia subjects carrying the Val/Val genotype had significantly reduced chemotaxis compared with those carrying the Met/Met genotype ($P = 0.0034$).

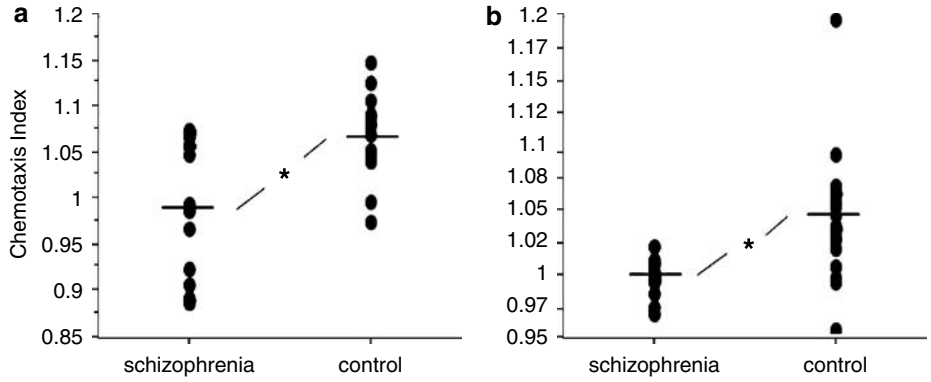

Figure 5 NRG1-induced migration in patients and controls. (**a**) The first cohort study: NRG1α-induced cell migration of B lymphoblasts from individuals with schizophrenia ($n=13$) and controls ($n=13$). Cells were plated in the upper chamber of a Transwell filter with 8 μm pores and allowed to migrate for 4 h in the presence or absence of NRG1α (200 ng/ml). Bars = median. *$P=0.007$, patients vs. control subjects. (**b**) The second independent cohort study: NRG1γ-induced cell migration of B lymphoblasts from individuals with schizophrenia ($n=12$) and controls ($n=20$). Cells were plated in the upper chamber of a Transwell filter with 8 μm pores and allowed to migrate for 4 h in the presence or absence of NRG1α (200 ng/ml). Bars = median. *$P=0.0006$, patients vs control subjects.

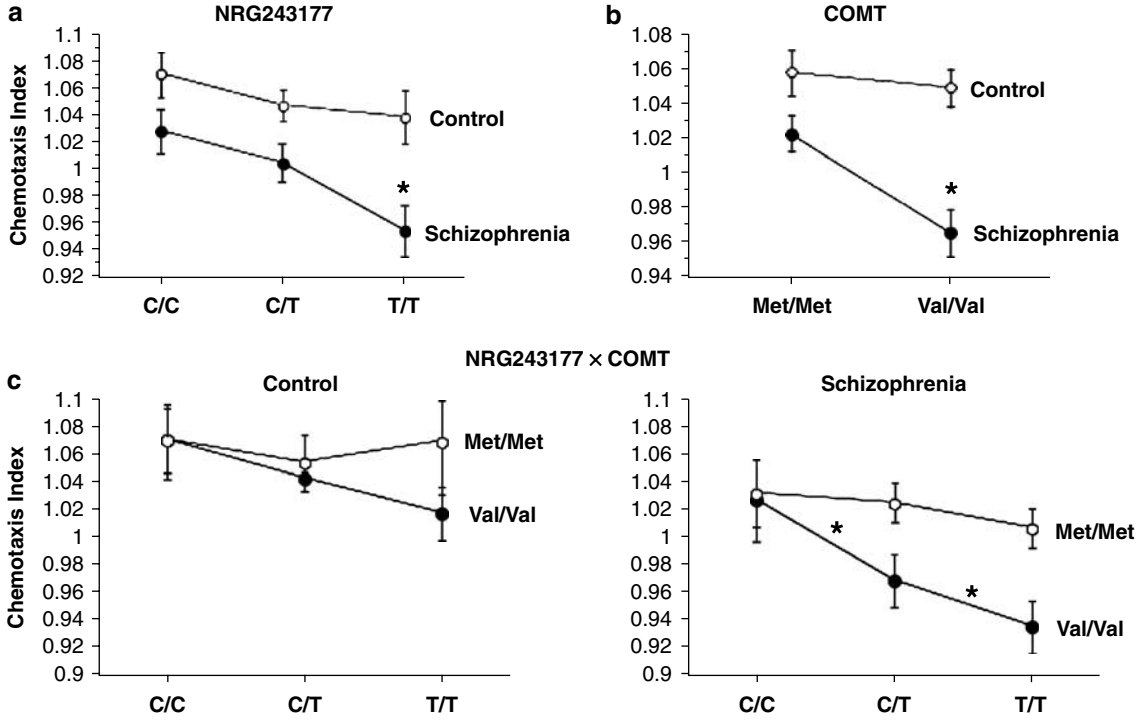

Figure 6 Genotype effects on NRG1-induced migration in the combined cohorts. (**a**) NRG243177 C/T genotype and NRG1-induced migration (chemotaxis index, mean $\pm$ s.e.). *$P=0.006$, C/C vs T/T; $P=0.02$, C/T vs T/T (see Results). The number of subjects carrying C/C, C/T and T/T genotype was 11, 16 and 5, respectively, in control subjects and 5, 12 and 7, respectively in schizophrenic patients. (**b**) COMT Val108/158Met genotype and NRG1-induced migration (chemotaxis index, mean $\pm$ s.e.m.). *$P=0.0034$, Val/Val vs Met/Met in patients with schizophrenia (see Results). The number of subjects carrying Met/Met and Val/Val was 14 and 19, respectively, in control subjects, and 13 and 12, respectively, in schizophrenic patients. (**c**) Effects of NRG243177 C/T and COMT Val108/158Met genotype on NRG1-induced migration (chemotaxis index, mean $\pm$ s.e.m.). *$P=0.024$, significant parametric relationship between the number of T alleles and chemotaxis in patients carrying COMT Val/Val (see Results). The number of subjects carrying each genotype was the same as for (**a**) and (**b**).

Finally, regression coefficients analysis indicated that there was a significant interaction between NRG243177 and COMT genotype ($P=0.0043$) (Figure 6c). This was owing to a significant parametric relationship between the number of T alleles of NRG243177 and chemotaxis ($P=0.0014$ in all subjects carrying COMT Val/Val and $P=0.024$ in patients with Val/Val) and a lack of the relationship in both subject

groups carrying COMT Met/Met (Figure 6c). As negative controls, we tested three other NRG1 SNPs, NRG221132, NRG221533, NRG241930, which have not been associated with effects on NRG1 expression, and none of these showed significant effect on NRG1-induced chemotaxis in the entire sample, or when schizophrenics and controls were considered separately. Covarying factors such as age and gender did not affect NRG1-induced chemotaxis ($P > 0.35$).

Discussion

Altered NRG1–ErbB signaling has been implicated as a causative mechanism in schizophrenia.[24,25] However, there has been no biological model to test the functional status of this system in living human subjects. In this study, we examined NRG1–ErbB signaling in patients with schizophrenia using EBV-transformed B lymphoblasts because they are easily established from patients and can be maintained to investigate various cellular and molecular biological functions. Previous studies have confirmed the expression of ErbB receptors in primary hematopoietic cells,[46] B cell leukemia-derived lymphoblasts[47,48] and multiple myeloma cells,[49] suggesting that hematopoietic cells, especially the B-cell lineage, express ErbB receptors. Consistent with these previous findings, we found that ErbB2 and ErbB3 transcripts and proteins were expressed in all the EBV-transformed B lymphoblasts tested. Immunohistochemical studies clearly demonstrated that ErbB2 and ErbB3 receptors are highly localized to the plasma membrane (Figure 2, Supplementary Figures S2 and S3) and redistributed in the form of receptor capping at the front edge of cells in response to extracellular exposure to NRG1 (Figure 2c and d). ErbB2 is a unique ligandless tyrosine kinase of the ErbB family that forms heterodimers with either ErbB3 or ErbB4 and provides a more potent signal than ErbB3 or ErbB4 alone.[50] Colocalization of ErbB2 and ErbB3 in response to NRG1 stimulation appears to be an indication of heterodimerization of these two receptors in our cells.

Activation of ErbB kinase by the co-localization of ErbB2 and ErbB3 receptors in response to NRG1 is evidenced by activation of Ras–MAPK, PI3K–AKT, and PLCγ–Ca^{2+} pathways. We show here that NRG1 induces phosphorylation of ERK at Tyr^{180}, phosphorylation of AKT at Ser^{473} and change in intracellular Ca^{2+} levels in a time-dependent manner (Figure 3 and Supplementary Figure S4). Moreover, we demonstrate that exogenous application of NRG1 induces cell migration (Figure 4a), which is a dynamic cell function mediated by ErbB kinase, confirmed here by the fact that the ErbB2-specific inhibitor AG825 blocked NRG1-mediated chemotaxis (Figure 4b). Furthermore, investigations using other kinase inhibitors indicated that NRG1-induced migration of B lymphoblasts also requires activations of PI3K/AKT and PLCγ (Figure 4c). Therefore, B lymphoblasts possess a functional NRG1–ErbB signaling system that is analogous to that observed in neuronal cells.[19,51] We used the α rather than β EGF-like domain because it is more representative of NRG1 in non-neuronal tissues; however, since the β form has been shown to be more potent than the α form in most assays and also thought to be the major bioactive form in the nervous system,[52] dose–response comparisons for various NRG1 isoforms would be of interest in a future study.

We next examined NRG1-induced cell migration in patients with schizophrenia and controls using two different transwells (5 and 8 μm pore size) and cell detection methods (CyQuant and Calcein AM, respectively). In both tests, we found a significant decrease in the chemotactic response of B lymphoblasts to NRG1 in patients with schizophrenia compared with that of control individuals (Figure 5a and Supplementary Figure S5). Subsequently, this was confirmed again in a second cohort of 32 subjects (Figure 5b). At present, we do not know the specific molecular mechanisms that underlie the impaired NRG1-mediated chemotaxis in schizophrenia. The use of transformed cells is a potential confounder, but we have observed no abnormalities in the general biologic characteristics of these cells from patients with schizophrenia, for example, replication times and non-specific movement. Because these cells are many generations removed from their natural progenitors, it is doubtful that environmental artifacts (e.g., effects of nutrition or medical treatments) would play a role. We have not found clear differences in expression and co-localization status of ErbB2 and ErbB3, or activation of Ras–MAPK and PLCγ signaling between control and schizophrenic individuals. However, NRG1-induced phosphorylation of AKT1 was decreased in at least a subset of patients with schizophrenia compared with that in controls. Thus, impaired NRG1-induced cell migration could be due in part to impaired PI3K–AKT signaling. Emamian *et al*.[53] have recently reported reduced AKT1–glycogen synthase kinase (GSK)-3β signaling in B lymphoblasts and brains of individuals with schizophrenia, which was apparently caused by reduced AKT1 protein expression. However, we found no reduction in the amount of AKT1 protein in our samples (Supplementary Figure S6). Further investigation is needed to determine the role of PI3K–AKT signaling in impaired NRG1-induced cell migration in schizophrenia.

An important possibility suggested by our data is that molecular defects, which may be either genetic, epigenetic, or both, are preserved in B lymphoblasts from patients with schizophrenia. In this regard, the T allele in SNP8NRG243177, the risk allele in the deCode haplotype at this SNP, is here associated with lesser NRG1-mediated migration compared with the C allele in patients with schizophrenia (Figure 6a). While this genetic association was not significant in the control subjects, the tendency for T alleles to predict less migration was observed and there was no significant interaction of genotype and diagnosis. Although the functional impact of schizophrenia-associated SNPs in the NRG1 gene remains unclear,

some of them may modulate the pharmacological ligand–receptor relationship by altering levels of different alternative splice forms of NRG1.[52] Interestingly, Law *et al*.[44] recently reported that there were substantial differences in expression of NRG1 type IV transcripts in brains from schizophrenics between the T allele in NRG243177 and C allele groups. Moreover, the same allele at this SNP has recently been shown to be associated with decreased expression of the $\alpha 7$ acetylcholine receptor (Mathew *et al.*, 2005; program number 675.18, Society for Neuroscience), a receptor previously shown to be regulated by NRG1.[23] Thus, converging evidence from several independent lines of investigation suggests that NRG243177 is a functional SNP in a regulatory domain of NRG1 that impacts on NRG1 function. Indeed, a bioinformatics analysis of this SNP indicates that it alters binding of transcription factors implicated in NRG1 regulation.[44]

It is not clear why a polymorphism in NRG1 would affect NRG1-induced migration. The NRG243177 SNP presumably differentially affects NRG1 expression, but it is yet to be determined how putative differences in NRG1 production by lymphocytes would influence their response to exogenously applied NRG1. One possibility is that autocrine NRG1–ErbB signaling, which has been found in other cells,[54] might lead to ErbB desensitization and to changes in back-signaling,[55] although this possibility has not yet been investigated in our cell system. In this regard, the association of genotype and NRG1-mediated migration in patients with schizophrenia may reflect the fact that their cells show impaired migration in response to this ligand, as a consequence of other molecular alterations, such as impairment of AKT activation.

Because schizophrenia is a polygenic and genetically heterogeneous condition,[24] it is possible that other genes converge on basic mechanisms of susceptibility. To the extent that NRG1-induced cell migration may underlie the association of NRG1 with schizophrenia, we tested whether another gene might also impact on this model. We chose the COMT Val^{158}Met polymorphism for three reasons: (1) it is linked to psychosis;[24] (2) it is a functional polymorphism and not a marker allele and as such its biologic association should be undiluted; (3) it has been implicated as a factor in another cell migration phenomenon, metastatic cancer.[45] Indeed, our findings demonstrate that the functional COMT Val^{158}Met polymorphism influences NRG1-mediated cell migration. The COMT enzyme is responsible for dopamine catabolism and has been shown to influence cortical dopaminergic signaling and function, especially in prefrontal cortex, presumably since dopamine transporters are relatively scarce in this region.[24,56] A common valine-to-methionine substitution in the COMT gene, Val^{158}Met, leads to a significant reduction in the enzyme's activity and influence dopaminergic signaling.[57] It is widely accepted that the high activity Val allele is associated with impaired cortical function in a number of neuropsychiatric phenotypes, presumably owing to greater dopamine catabolism, and may be implicated as a schizophrenia risk allele.[24] Consistent with the hypothesis that Val is a risk allele, the subjects carrying the Val/Val genotype evinced significantly lower NRG1-mediated migration compared with those carrying Met/Met (Figure 6b). Moreover, severe defects in NRG1-stimulated phosphorylation of AKT were observed at least in some subjects carrying Val/Val (Supplementary Figure S7). These results suggest a novel biological effect of COMT potentially related to schizophrenia pathogenesis, and indicate an epistatic interaction of these genes at a biological level. Indeed, another striking fact is that there is a significant gene-to gene interaction between COMT and NRG1 genes (Figure 6c). It is generally assumed that multiple genes interact with each other and with the environment to account for the risk architecture of schizophrenia,[24] but to our knowledge, this is the first demonstration of biologic epistasis between putative schizophrenia risk genes. Thus, the effect of the T allele in NRG243177 on NRG1-induced migration is significant in the COMT Val homozygous group, but not in the Met homozygous group. These findings implicate complex interactions of susceptibility genes for schizophrenia in mechanisms underlying NRG1-induced cell migration.

NRG1–ErbB signaling has been found to play critical regulatory roles in the migration of neuronal precursors along radial glial fibers. In the developing central nervous system, NRG1–ErbB signaling is essential for induction of the elongation of cortical radial glia fibers, and migration of cortical neurons and cerebellar granule cells.[26,27] If common genes shared by neuronal cells and lymphocytes regulate NRG1-induced migration, our findings in B lymphoblasts from schizophrenia suggest that NRG1-mediated neural migration may be abnormal in patients with schizophrenia during development. Although controversial, aberrant neuronal migration has been suspected from reports of cytoarchitectural abnormalities in the cortex of schizophrenic patients.[58] Thus, our findings suggest a potential molecular pathway involved in the neurodevelopmental origins of schizophrenia.[34,38]

Acknowledgments

This research was supported by the Intramural Research Program of the National Institute of Mental Health, NIH. We thank Drs SP Markey and DL Murphy, NIMH, for their generous support, Drs CA Combs at the National Heart, Lung, and Blood Institute and C Smith at the National Institute of Neurological Disorders and Stroke for assistance with confocal microscopy, and DJ Venzon of the National Cancer Institute for statistical consultation and advice. We also thank Drs JW Daly of the National Institute of Diabetes and Digestive and Kidney Diseases, L Neckers of the National Cancer Institute and B Lu of the National Institute of Child Health and

Human Development or scientific advice and critical review of the manuscript.

References

1 Pulver AE, Lasseter VK, Kasch L, Wolyniec P, Nestadt G, Blouin JL *et al.* Schizophrenia: a genome scan targets chromosomes 3p and 8p as potential sites of susceptibility genes. *Am J Med Genet* 1995; **60**: 252–260.
2 Kendler KS, MacLean CJ, O'Neill FA, Burke J, Murphy B, Duke F *et al.* Evidence for a schizophrenia vulnerability locus on chromosome 8p in the Irish Study of High-Density Schizophrenia Families. *Am J Psychiatry* 1996; **153**: 1534–1540.
3 Kaufmann CA, Suarez B, Malaspina D, Pepple J, Svrakic D, Markel PD *et al.* NIMH Genetics Initiative Millenium Schizophrenia Consortium: linkage analysis of African-American pedigrees. *Am J Med Genet* 1998; **81**: 282–289.
4 Blouin JL, Dombroski BA, Nath SK, Lasseter VK, Wolyniec PS, Nestadt G *et al.* Schizophrenia susceptibility loci on chromosomes 13q32 and 8p21. *Nat Genet* 1998; **20**: 70–73.
5 Shaw SH, Kelly M, Smith AB, Shields G, Hopkins PJ, Loftus J *et al.* A genome-wide search for schizophrenia susceptibility genes. *Am J Med Genet* 1998; **81**: 364–376.
6 Brzustowicz LM, Honer WG, Chow EW, Little D, Hogan J, Hodgkinson K *et al.* Linkage of familial schizophrenia to chromosome 13q32. *Am J Hum Genet* 1999; **65**: 1096–1103.
7 Badner JA, Gershon ES. Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. *Mol Psychiatry* 2002; **7**: 405–411.
8 Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I *et al.* Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. *Am J Hum Genet* 2003; **73**: 34–48.
9 Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S *et al.* Neuregulin 1 and susceptibility to schizophrenia. *Am J Hum Genet* 2002; **71**: 877–892.
10 Stefansson H, Sarginson J, Kong A, Yates P, Steinthorsdottir V, Gudfinnsson E *et al.* Association of neuregulin 1 with schizophrenia confirmed in a Scottish population. *Am J Hum Genet* 2003; **72**: 83–87.
11 Williams NM, Preece A, Spurlock G, Norton N, Williams HJ, Zammit S *et al.* Support for genetic variation in neuregulin 1 and susceptibility to schizophrenia. *Mol Psychiatry* 2003; **8**: 485–487.
12 Law AJ, Weickert CS, Hyde TM, Kleinman JE, Harrison PJ. Neuregulin-1 (NRG-1) mRNA and protein in the adult human brain. *Neuroscience* 2004; **127**: 125–136.
13 Hashimoto R, Straub RE, Weickert CS, Hyde TM, Kleinman JE, Weinberger DR. Expression analysis of neuregulin-1 in the dorsolateral prefrontal cortex in schizophrenia. *Mol Psychiatry* 2004; **9**: 299–307.
14 Aston C, Jiang L, Sokolov BP. Microarray analysis of postmortem temporal cortex from patients with schizophrenia. *J Neurosci Res* 2004; **77**: 858–866.
15 Rimer M, Barrett DW, Maldonado MA, Vock VM, Gonzalez-Lima F. Neuregulin-1 immunoglobulin-like domain mutant mice: clozapine sensitivity and impaired latent inhibition. *NeuroReport* 2005; **16**: 271–275.
16 Gerlai R, Pisacane P, Erickson S. Heregulin, but not ErbB2 or ErbB3, heterozygous mutant mice exhibit hyperactivity in multiple behavioral tasks. *Behav Brain Res* 2000; **109**: 219–227.
17 Huang YZ, Won S, Ali DW, Wang Q, Tanowitz M, Du QS *et al.* Regulation of neuregulin signaling by PSD-95 interacting with ErbB4 at CNS synapses. *Neuron* 2000; **26**: 443–455.
18 Kwon OB, Longart M, Vullhorst D, Hoffman DA, Buonanno A. Neuregulin-1 reverses long-term potentiation at CA1 hippocampal synapses. *J Neurosci* 2005; **25**: 9378–9383.
19 Ozaki M, Sasner M, Yano R, Lu HS, Buonanno A. Neuregulin-beta induces expression of an NMDA-receptor subunit. *Nature* 1997; **390**: 691–694.
20 Gu Z, Jiang Q, Fu AK, Ip NY, Yan Z. Regulation of NMDA receptors by neuregulin signaling in prefrontal cortex. *J Neurosci* 2005; **25**: 4974–4984.
21 Rieff HI, Raetzman LT, Sapp DW, Yeh HH, Siegel RE, Corfas G. Neuregulin induces GABA(A) receptor subunit expression and neurite outgrowth in cerebellar granule cells. *J Neurosci* 1999; **19**: 10757–10766.
22 Okada M, Corfas G. Neuregulin1 downregulates postsynaptic $GABA_A$ receptors at the hippocampal inhibitory synapse. *Hippocampus* 2004; **14**: 337–344.
23 Liu Y, Ford B, Mann MA, Fischbach GD. Neuregulins increase alpha7 nicotinic acetylcholine receptors and enhance excitatory synaptic transmission in GABAergic interneurons of the hippocampus. *J Neurosci* 2001; **21**: 5660–5669.
24 Harrison PJ, Weinberger DR. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. *Mol Psychiatry* 2005; **10**: 40–68.
25 Corfas G, Roy K, Buxbaum JD. Neuregulin 1–ErbB signaling and the molecular/cellular basis of schizophrenia. *Nat Neurosci* 2004; **7**: 575–580.
26 Anton ES, Marchionni MA, Lee KF, Rakic P. Role of GGF/neuregulin signaling in interactions between migrating neurons and radial glia in the developing cerebral cortex. *Development* 1997; **124**: 3501–3510.
27 Rio C, Rieff HI, Qi P, Khurana TS, Corfas G. Neuregulin and erbB receptors play a critical role in neuronal migration. *Neuron* 1997; **19**: 39–50.
28 Flames N, Long JE, Garratt AN, Fischer TM, Gassmann M, Birchmeier C *et al.* Short- and long-range attraction of cortical GABAergic interneurons by neuregulin-1. *Neuron* 2004; **44**: 251–261.
29 Lopez-Bendito G, Cautinat A, Sanchez JA, Bielle F, Flames N, Garratt AN *et al.* Tangential neuronal migration controls axon guidance: a role for neuregulin-1 in thalamocortical axon navigation. *Cell* 2006; **125**: 127–142.
30 Calaora V, Rogister B, Bismuth K, Murray K, Brandt H, Leprince P *et al.* Neuregulin signaling regulates neural precursor growth and the generation of oligodendrocytes *in vitro*. *J Neurosci* 2001; **21**: 4740–4751.
31 Sussman CR, Vartanian T, Miller RH. The ErbB4 neuregulin receptor mediates suppression of oligodendrocyte maturation. *J Neurosci* 2005; **25**: 5757–5762.
32 Colognato H, Ramachandrappa S, Olsen IM, ffrench-Constant C. Integrins direct Src family kinases to regulate distinct phases of oligodendrocyte development. *J Cell Biol* 2004; **167**: 365–375.
33 Kim JY, Sun Q, Oglesbee M, Yoon SO. The role of ErbB2 signaling in the onset of terminal differentiation of oligodendrocytes *in vivo*. *J Neurosci* 2003; **23**: 5561–5571.
34 Weinberger DR, Marenco S. Schizophrenia as a neurodevelopmental disorder. In: Hirsch S, Weinberger DR (eds). *Schizophrenia*. Blackwell Science: Oxford, UK, 2003, pp 326–348.
35 Wu JY, Feng L, Park HT, Havlioglu N, Wen L, Tang H *et al.* The neuronal repellent Slit inhibits leukocyte chemotaxis induced by chemotactic factors. *Nature* 2004; **410**: 948–952.
36 Ma Q, Jones D, Borghesani PR, Segal RA, Nagasawa T, Kishimoto T *et al.* Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. *Proc Natl Acad Sci USA* 1998; **95**: 9448–9453.
37 Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. *Nature* 1998; **393**: 595–599.
38 Weinberger DR. Implications of normal brain development for the pathogenesis of schizophrenia. *Arch Gen Psychiatry* 1987; **44**: 660–669.
39 Egan MF, Goldberg TE, Gscheidle T, Weirich M, Bigelow LB, Weinberger DR. Relative risk of attention deficits in siblings of patients with schizophrenia. *Am J Psychiatry* 2000; **157**: 1309–1316.
40 Pressman S, Rotter JI. Epstein–Barr virus transformation of cryopreserved lymphocytes: prolonged experience with technique. *Am J Hum Genet* 1991; **49**: 467.
41 Kato S, Kobayashi T, Yamada K, Nishii K, Sawada H, Ishiguro H *et al.* Expression of erbB receptors mRNA in thyroid tissues. *Biochim Biophys Acta* 2004; **1673**: 194–200.
42 Kriebel PW, Barr VA, Parent CA. Adenylyl cyclase localization regulates streaming during chemotaxis. *Cell* 2003; **112**: 549–560.
43 Marmor MD, Skaria KB, Yarden Y. Signal transduction and oncogenesis by ErbB/HER receptors. *Int J Radiat Oncol Biol Phys* 2004; **58**: 903–913.

44 Law AJ, Lipska BK, Weickert CS, Hyde TM, Straub RE, Hashimoto R *et al.* Neuregulin 1 transcripts are differentially expressed in schizophrenia and regulated by 5′ SNPs associated with the disease. *Proc Natl Acad Sci USA* 2005; **103**: 6747–6752.
45 Matsui A, Ikeda T, Enomoto K, Nakashima H, Omae K, Watanabe M *et al.* Progression of human breast cancers to the metastatic state is linked to genotypes of catechol-*O*-methyltransferase. *Cancer Lett* 2000; **150**: 23–31.
46 Leone F, Perissinotto E, Cavalloni G, Fonsato V, Bruno S, Surrenti N *et al.* Expression of the c-ErbB-2/HER2 proto-oncogene in normal hematopoietic cells. *J Leukoc Biol* 2003; **74**: 593–601.
47 Roy-Burman P, Devi BG, Parker JW. Differential expression of c-erbB, c-myc and c-myb oncogene loci in human lymphomas and leukemias. *Int J Cancer* 1983; **32**: 185–191.
48 Buhring HJ, Sures I, Jallal B, Weiss FU, Busch FW, Ludwig WD *et al.* The receptor tyrosine kinase p185HER2 is expressed on a subset of B-lymphoid blasts from patients with acute lymphoblastic leukemia and chronic myelogenous leukemia. *Blood* 1995; **86**: 1916–1923.
49 Mahtouk K, Hose D, Reme T, De Vos J, Jourdan M, Moreaux J *et al.* Expression of EGF-family receptors and amphiregulin in multiple myeloma. Amphiregulin is a growth factor for myeloma cells. *Oncogene* 2005; **24**: 3512–3524.
50 Graus-Porta D, Beerli RR, Daly JM, Hynes NE. ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling. *EMBO J* 1997; **16**: 1647–1655.
51 Buonanno A, Fischbach GD. Neuregulin and ErbB receptor signaling pathways in the nervous system. *Curr Opin Neurobiol* 2001; **11**: 287–296.
52 Falls DL. Neuregulins: functions, forms, and signaling strategies. *Exp Cell Res* 2003; **284**: 14–30.
53 Emamian ES, Hall D, Birnbaum MJ, Karayiorgou M, Gogos JA. Convergent evidence for impaired AKT1–GSK3beta signaling in schizophrenia. *Nat Genet* 2004; **36**: 131–137.
54 Li Q, Ahmed S, Loeb JA. Development of an autocrine neuregulin signaling loop with malignant transformation of human breast epithelial cells. *Cancer Res* 2004; **64**: 7078–7085.
55 Bao J, Wolpowitz D, Role LW, Talmage DA. Back signaling by the Nrg-1 intracellular domain. *J Cell Biol* 2003; **161**: 1133–1141.
56 Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE *et al.* Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci USA* 2001; **98**: 6917–6922.
57 Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S *et al.* Functional analysis of genetic variation in catechol-*O*-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Hum Genet* 2004; **75**: 807–821.
58 Harrison PJ. The neuropathology of schizophrenia. A critical review of the data and their interpretation. *Brain* 1999; **122**: 593–624.

Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)