Genetic Variation in Neuregulin I is Associated with Differences in Prefrontal Engagement in Children

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Abstract: The majority of psychopathology is rooted early in life and first emerges during childhood and adolescence. However, little is known about how risk genes affect brain function to increase biological vulnerability to psychopathology in childhood, because most imaging genetic studies published so far have been conducted on adult participants. We examined the impact of neuregulin1 (NRG1), a probable susceptibility gene for schizophrenia and bipolar disorder, on brain function in a sample of 102 ten- to twelve-year-old children. Each participant performed a Go/Nogo task, whereas brain responses were measured using functional magnetic resonance imaging. Statistical parametric mapping was used to estimate the impact of genetic variation in NRG1 on brain activation. Response accuracy and reaction times did not differ as a function of NRG1 genotype. However, individuals with the high-risk variant expressed greater brain activation for both Go and Nogo stimuli in the right posterior orbital gyrus, where NRG1 genotype accounted for 11% of interindividual variance. There were no regions showing a significant interaction between NRG1 genotype and stimulus type even at trend level, suggesting that the impact of NRG1 on brain activation was not specific to either response inhibition or motor execution. These results suggest that that genetic variation in NRG1 is associated with different levels of prefrontal engagement in children as young as 10–12 years of age. Our investigation provides support to the idea that genetic factors may affect brain function to moderate vulnerability to psychopathology from childhood. *Hum Brain Mapp* 30:3934–3943, 2009. © 2009 Wiley-Liss, Inc.

Key words: neuregulin1; schizophrenia; bipolar disorder; childhood; Go/Nogo task; functional magnetic resonance imaging

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INTRODUCTION

Schizophrenia and bipolar disorder are severe psychiatric disorders with a strong genetic component. In recent years, imaging genetic studies have demonstrated the effects of several schizophrenia and bipolar disorder risk genes on brain structure and function in adult participants [Fisher et al., 2008]. Given that very few psychiatric illnesses arise “de novo” in adulthood [Kim-Cohen et al., 2003]; however, it is important to extend the current imaging genetic work to include child samples. The aim of this

study is to investigate the mechanisms by which genetically influenced differences in brain function in childhood could predispose to mental illness during later development. In particular, we used functional magnetic resonance imaging (fMRI) to examine the effects of Neuregulin1 (NRG1), a promising candidate gene for schizophrenia and bipolar disorder, on brain responses during executive control processing in children of 10–12 years of age.

NRG1, located at 8p21-p22, was originally associated with schizophrenia by Stefansson et al. [2002, 2003], who found association of a so-called core haplotype in the NRG1 gene with a twofold increased risk for schizophrenia in Icelandic and Scottish populations. Further evidence has since come from a series of studies in Irish, Chinese and South African, mixed British and Dutch samples using the same haplotype region [Tosato et al., 2005], as well as meta-analysis [Li et al., 2006]. More recently, two independent molecular genetic studies [Green et al., 2005; Thomson et al., 2007] have implicated NRG1 in bipolar disorder. Taken collectively, these studies suggest that NRG1 may confer biological susceptibility to psychopathology across the traditional Kraepelian dichotomy of affective and nonaffective psychoses [Craddock et al., 2007].

The functional significance of the core risk haplotype or any of its markers is currently unknown and it is possible that as yet unidentified mutations in linkage disequilibrium (LD) with it are also responsible for a functional effect. The NRG1 gene spans 1.2 Mb and encodes many structurally and functionally distinct protein isoforms through alternative splicing, grouped in I–IV types, from which type I–III isoforms are known to participate in ErbB signaling [Falls, 2003; Steinthorsdottir et al., 2004]. These proteins have been implicated in a diverse range of functions, including neuronal migration and specification, oligodendrocyte differentiation and myelination, hormonal control of puberty, regulation of acetylcholine and expression of glutamate, GABA and other neurotransmitter receptors [Corfas et al., 2004]. Disruption of these developmental processes has also been implicated, directly and indirectly, in mental illness [Corfas et al., 2004]. Two recent investigations in mice [Li et al., 2007; Woo et al., 2007] have provided evidence that NRG1 and its receptor erbB4 regulate transmission at brain glutamate and GABA synapses in hippocampal and prefrontal regions, consistent with the notion of synaptic defects in schizophrenia and bipolar disorder.

Four recent neuroimaging investigations have examined the impact of NRG1 genotype on brain function in adult participants. First, Hall et al. [2006] found that relatives of people with schizophrenia with the risk allele of the NRG1 gene show reduced medial prefrontal and temporo-occipital activation during a sentence completion task compared to relatives without the risk allele. Krug et al. [2008] subsequently reported a positive dose-dependent effect of the risk allele on prefrontal activation in healthy individuals

during a working memory task. In contrast, Kircher et al. [2008] found that in patients with first episode schizophrenia who were performing a working memory task, the risk variant was associated with reduced activation in the hippocampus, precuneus, cerebellum, and anterior cingulate. Finally, Mechelli et al. [2008] found differential effects of NRG1 genotype on prefrontal function in healthy volunteers and patients with schizophrenia and bipolar disorder using a verbal fluency task. In all the three diagnostic groups, the high-risk variant of NRG1 was associated with greater deactivation of the left precuneus; in addition, the high-risk variant was associated with increased activation of the right inferior frontal gyrus, but only in patients with schizophrenia, and the right posterior orbital gyrus, but only in patients with bipolar disorder [Mechelli et al., 2008]. These results indicate that genetic variation in NRG1 has a measurable impact on brain function during executive cognitive processing and furthermore provide evidence for a disease-specific pattern of gene action in different regions of the prefrontal cortex.

It is currently unclear, however, whether the effects of NRG1 genotype on brain responses that have been reported in adult participants are also evident in children. This is an important question because there is an increasing evidence that the majority of psychopathology is rooted early in life and first emerges during childhood and adolescence [Kim-Cohen et al., 2003]. If gene-related differences in adult participants reflect alterations that occurred during childhood and adolescence, then a better characterization of these developmental alterations is critical for understanding how genes affect brain structure and function to mediate vulnerability to psychopathology [Viding et al., 2006]. A recent investigation on the impact of NRG1 genotype on brain structure demonstrated that the effect of this gene on fronto-temporal gray matter volume is already evident during adolescence and significantly differs between patients with childhood onset schizophrenia and healthy controls [Addington et al., 2007]. However, no previous studies have examined the effects of NRG1 genotype on brain responses in children.

We therefore used fMRI to explore the impact of NRG1 genotype in 102 children aged between 10 and 12 using a Go/Nogo paradigm which required subjects to either press, or refrain from pressing, a designated button when presented with a Go or Nogo stimulus, respectively. The use of the Go/Nogo paradigm in conjunction with a resting condition allows the identification of regions which are engaged during response inhibition (“Nogo”) and response execution (“Go”) relative to resting as well as regions that express differential activation for response inhibition compared to motor execution [Menon et al., 2001]. The Go/Nogo paradigm was chosen because it engages prefrontal brain regions and executive control processes which are known to be adversely affected in both schizophrenia and bipolar disorder [Altshuler et al., 2005; Arce et al., 2006; Bark et al., 2005; Ford et al., 2004; Kaladjian

TABLE I. Participants' characteristics and task performance

	CC	CT	TT	All subjects	P-value
N	43	52	7	102	
Age in months	135.7 (7.4)	136.9 (8.5)	130.0 (5.7)	135.8 (8.10)	0.10
Full scale IQ	104.6 (13.7)	102.5 (11.6)	105.4 (7.5)	103.3 (12.2)	0.58
Parent rated SDQ scores					
Hyperactivity	3.23 (2.70)	3.96 (2.63)	2.14 (1.95)	3.52 (2.65)	0.47
Conduct problems	1.93 (1.84)	1.46 (1.44)	1.71 (2.56)	1.67 (1.70)	0.62
Emotional problems	1.41 (1.63)	1.57 (1.85)	1.00 (1.29)	1.47 (1.72)	0.96
Prosociality	8.25 (1.67)	8.19 (2.14)	8.85 (1.34)	8.26 (1.90)	0.12
Peer problems	1.09 (1.71)	1.03 (1.70)	2.57 (4.15)	1.16 (1.97)	0.53
Total difficulties	7.67 (5.89)	7.96 (5.50)	7.42 (7.02)	7.80 (5.72)	0.54
Teacher rated SDQ scores					
Hyperactivity	3.13 (2.78)	4.69 (3.00)	0.71 (1.11)	3.73 (3.01)	<0.0001
Conduct problems	1.28 (2.00)	1.93 (2.49)	0.85 (1.21)	1.58 (2.23)	0.21
Emotional problems	1.25 (1.66)	1.66 (1.98)	1.14 (1.67)	1.45 (1.83)	0.55
Prosociality	6.54 (3.02)	6.38 (2.92)	7.85 (2.73)	6.55 (2.94)	0.42
Peer problems	1.44 (2.39)	1.02 (1.93)	2.14 (3.53)	1.28 (2.26)	0.47
Total difficulties	7.07 (6.33)	9.24 (6.82)	4.85 (7.03)	8.00 (6.70)	0.14
Performance on the Go/Nogo task					
Errors for Go trials (misses)	2.25 (4.15)	1.82 (3.08)	2.00 (1.29)	2.01 (3.48)	0.83
Errors for Nogo trials (false alarms)	3.65 (2.36)	3.92 (3.19)	5.85 (4.22)	3.94 (2.97)	0.19
RT for Go trials	394.67 (38.25)	398.23 (40.50)	379.10 (60.83)	395.41 (40.98)	0.50

Data are expressed as mean values and standard deviation. *P*-values refer to a one-way ANOVA contrasting the different genotypes. N, number of subjects; SDQ, Strengths and Difficulties Questionnaire; RT, reaction times.

et al., 2007; Rubia et al., 2001]. For example, patients with schizophrenia express reduced activation of the left anterior cingulate gyrus and the right ventro-lateral prefrontal-cortex, particularly during response inhibition ("Nogo") compared to motor execution ("Go") [Kaladjian et al., 2007; Rubia et al., 2001]; similarly, patients with bipolar disorder show blunted activation of the right orbitofrontal cortex, right cingulate cortex, and right hippocampus for Nogo minus Go stimuli [Altshuler et al., 2005]. We tested the hypothesis that NRG1 genotype had a measurable impact on brain function in our sample of children, consistent with the notion that genetic factors affect brain function to moderate vulnerability to psychopathology from an early age [Viding et al., 2006]. One possibility was that the impact of NRG1 would be greater for Nogo than Go stimuli; this would indicate that the impact of this gene is more pronounced during response inhibition than motor execution, consistent with the functional alterations observed in patients with schizophrenia and bipolar disorder. An alternative possibility was that the impact of NRG1 on brain function would be expressed similarly for Go and Nogo trials relative to resting; this would suggest that genetic variation in NRG1 has a more general impact on brain function and in particular affects one or more cognitive processes that are shared by the Go and Nogo conditions, such as visual processing, online monitoring of the stimuli, evaluation of their content, and selection of an appropriate response.

MATERIALS AND METHODS

Subjects

A total of 102 ten- to twelve-years-old boys participated in this study. Participants were recruited from the longitudinal Twins Early development Study (TEDS) database as part of an on-going twin neuroimaging project which included typically developing children ($n = 69$) as well as children in the top 10% of the U.K. population for conduct problems ($n = 33$). Crucially for the interpretation of the present data, the mean sample score on conduct problems or associated hyperactivity was neither in the abnormal range, nor did our sample have elevated levels of any other psychopathology as measured by Strengths and Difficulties Questionnaire [Goodman et al. 2000]. This was the case for both parent and teacher ratings (see Table I). None of the children, including those typically developing as well as those in the top 10% of the U.K. population for conduct problems, had an official conduct disorder diagnosis. Our sample included 18 pairs of monozygotic twins, 24 pairs of dizygotic twins, and 18 singletons, to account for the nonindependence of the twin data, statistical inferences in the present investigation were based on estimates of the standard errors corrected for clustering on family (see below). All participants had normal or corrected to normal vision, and none had a history of diagnosed neurological or neuropsychiatric problems. Written consent was

obtained from all participants and their parents in accordance with protocols approved by Institute of Psychiatry Ethics Committee.

Participants were genotyped for SNP8NRG243177 (see below) with C and T being the low-risk and high-risk alleles, respectively. This SNP was chosen because (i) it showed a significant association in Steffansson's original Icelandic study [Steffansson et al., 2002]; (ii) it was associated with changes in mRNA expression in a recent investigation [Law et al., 2006]; (iii) it has been shown to have a significant impact on brain structure and function in previous neuroimaging studies on adult participants [Hall et al., 2006; McIntosh et al., 2007]. The sample consisted of 43 individuals with the CC variant, 52 individuals with the CT variant, and 7 individuals with the TT variant. The short version of the Wechsler Abbreviated Scales of Intelligence (WASI) was used to assess IQ [Wechsler, 1999]. In addition, the Strengths and Difficulties Questionnaire was used to measure conduct problems, emotional problems, and hyperactivity in all participants [Goodman et al., 2000]. The three genotypic groups were compared using a regression analysis that accounted for the nonindependence of twin data, using STATA software (version 10). Demographic, cognitive, and behavioral data are summarized for each genotype group in Table I.

Genotyping

DNA was extracted from blood or cheek swabs using standard methods. Genotyping of the single nucleotide polymorphism SNP8NRG243177 (rs6994992) was performed using a competitive allele specific PCR system (CASP).

Go/Nogo Task

Participants were presented with pictures of "Spiderman" or "Hobgoblin," representing the Go and Nogo stimuli, respectively. Participants were instructed to respond as quickly as possible in response to the Go stimulus and to withhold response on the appearance of the Nogo stimulus. The data were acquired in one acquisition run lasting 4 min 48 s. A blocked design was used to present a total of 120 stimuli including 90 Go stimuli ("Spiderman" images) and 30 Nogo stimuli ("Hobgoblin" images). Three of the blocks comprised of 20 Go stimuli; the remaining three blocks comprised of 10 Go stimuli and 10 Nogo stimuli randomly intermixed. Stimulus duration was 1.5 s and stimulus rate was 40 stimuli per minute. Three periods of rest, each lasting 34 s, were also included during which participants were instructed to focus on a fixation cross in the middle of the screen.

fMRI Data Acquisition

A total of 144 functional image volumes measuring blood oxygenation level dependent (BOLD) signal were collected from each subject using T2*-weighted gradient echo-planar imaging sequence (EPI) with a repetition time

(RT) of 2 s (number of slices: 28; slice thickness 3.5 mm, gap = 0.3 mm; TE = 25 ms, field of view = 220 × 220, matrix size 64 × 64). Stimuli was projected onto a high-resolution screen located in front of the participant's head and were viewed via mirror attached to the head coil.

fMRI Data Analysis

The analysis of the fMRI data was performed using SPM5 software [Friston, 2003], running under Matlab 6.5. In brief, all volumes from each subject were realigned, normalized to a template and spatially smoothed with a 6 mm full width at half maximum isotropic Gaussian kernel. There are a number of studies providing evidence that atlas-transformed brain morphology is relatively consistent between 7- and 8-year-old children and adults at a resolution appropriate to fMRI [Burgund et al., 2002; Kang et al., 2003]; thus, the standard MNI-305 template was used for spatial normalization, consistent with several previous functional neuroimaging investigations of children (e.g. Bitan et al., 2009). First, the statistical analysis of regional responses was performed in a subject-specific fashion, by convolving each onset time with a synthetic hemodynamic response function (HRF). Although the stimuli were presented using a blocked design, the data were analyzed using an event-related model in which each trial was modeled as a single event. This allowed us to model correct and incorrect trials separately and obtain a more precise characterization of neuronal responses [Mechelli et al., 2003]. The resulting design matrix included a total of three conditions are as follows: (i) Go, (ii) Nogo, and (iii) incorrect responses. To remove low-frequency drifts, the data were high-pass filtered using a set of discrete cosine basis functions with a cutoff period of 128 s. After the parameter estimates were calculated for all brain voxels using the general linear model, contrast images were computed for each subject independently. Second, the subject-specific contrast images were entered into a full-factorial ANOVA to permit inferences at the second level [Penny and Holmes, 2003]. This allowed us to examine the impact of the experimental task on brain activation, the main effects of stimulus type (Go vs. Nogo), the main effect of genotype and their interaction. Because the superior regions of the prefrontal and parietal cortices were not covered in a subset of subjects because of suboptimal positioning in the scanner, these regions were excluded from the statistical analysis; the area of the brain included ranged from $z = -34$ to $z = 52$. Statistical inferences were made in SPM5 using a statistical threshold of $P < 0.05$ with family-wise error (FWE) correction for multiple comparisons and an extent threshold of 10 voxels.

Nonindependence of Twin Data

We used two complementary strategies to minimize the risk that our results would be biased by the presence of twins in our sample. The first strategy involved extracting the individual parameter estimates from the peak voxel of

all regions which expressed significant effects of NRG1 genotype and then using STATA software (version 10) to compare the different genotypic groups based on estimates of the standard errors corrected for clustering on family. This procedure allowed us to examine whether the effects of NRG1 genotype identified with SPM were still significant after correction for the nonindependence of twin data. In regions where there was a significant effect of genotype on brain function, we used the R^2 measure of effect size to assess how much of the interindividual variance in BOLD activation was explained by the genetic variation. The second strategy involved removing one twin from each pair and then repeating the statistical analysis using SPM software; this resulted in a sample of 60 participants consisting of 24 individuals with the CC variant, 30 individuals with the CT variant, and 6 individuals with the TT variant. This procedure allowed us to estimate the effects of NRG1 genotype on brain activation based on independent data.

RESULTS

Demographics

Table I reports demographic data for each genotypic group independently as well as for all subjects combined. In brief, the age and IQ of participants did not differ as a function of NRG1 genotype; furthermore, NRG1 genotype was not associated with parent or teacher ratings of conduct problems, emotional problems, prosociality, peer problems, and total difficulties. However, teacher rated hyperactivity differed as a function of NRG1 genotype ($F = 21.30$, $df = (2,56)$, $P < 0.0001$); post hoc t tests indicated lower scores in TT homozygotes compared to CC homozygotes ($P < 0.05$) as well as CT heterozygotes ($P < 0.05$).

Behavioral Performance

Analysis of response accuracy and reaction times was performed using STATA software. The three genotypic groups were compared using a regression analysis which accounted for the nonindependence of twin data. NRG1 genotype was not associated with number of "misses" ($F = 0.176$, $df = 2,99$, $P = 0.839$), number of "false alarms" ($F = 1.684$, $df = 2,99$, $P = 0.191$) or reaction times ($F = 0.680$, $df = 2,99$, $P = 0.509$). Table I reports response accuracy and reaction times for each genotypic group and for all subjects combined.

Go/Nogo Task

Effects of Go/Nogo Task Versus Fixation

A distributed network including bilateral frontal, temporal, parietal, and occipital areas expressed increased activation during Go and Nogo trials relative to fixation (see Fig. 1 and Table II).

Main Effect of Stimulus Type

The effect of stimulus type was examined by contrasting brain activation for Go and Nogo stimuli; this allowed us to identify areas which responded differentially to Go and Nogo stimuli on average across genotypes. Greater activation for Nogo relative to Go stimuli was detected in a right-lateralized network including the middle and inferior frontal cortex, the middle and inferior temporal cortex, the inferior parietal lobule, and the angular gyrus and the precuneus (see Fig. 1 and Table III). Conversely, greater activation for Go relative to Nogo stimuli was detected in a bilateral network including the cerebellum, fusiform gyrus, parahippocampal cortex, anterior cingulate gyrus, and insula (Fig. 1 and Table III).

Main Effect of Genotype

The main effect of genotype was examined by comparing the different genotype groups for Go and Nogo trials combined; this allowed us to identify areas that responded differentially to the different genotypes on average across Go and Nogo trials. We first performed an analysis which contrasted individuals with either one or two risk alleles ($n = 59$) against those without the risk allele ($n = 43$) resulting in a total of two genotypic groups. This revealed that individuals with the risk allele compared to those without it expressed increased activation in a region of the orbitofrontal cortex corresponding to the right posterior orbital gyrus ($x = 44$, $y = 24$, $z = -14$, z -score = 4.6, $P = 0.003$ after FWE correction) (see Fig. 2); in contrast, there were no areas expressing greater activation in individuals without the risk allele relative to those with either one or two risk alleles. We then performed an additional analysis which modeled individuals with one and two risk alleles separately resulting in a total of three genotypic groups. This second analysis revealed a significant difference between CC and TT homozygotes in the very same region identified by the first analysis ($x = 44$, $y = 24$, $z = -14$, z -score = 4.7, $P = 0.002$ after FWE correction). Exploration of the group-specific parameter estimates suggested a positive dose-dependent effect of the risk allele: in particular individuals with two risk alleles expressed significantly greater activation for Go and Nogo trials relative to fixation; in contrast individuals with one risk allele expressed a "trend" at uncorrected level (z -score = 3.1, $P = 0.001$ uncorrected); finally individuals without the risk allele did not express significantly greater activation for Go and Nogo trials relative to fixation even at trend level (see Fig. 2). There were no areas expressing a negative dose-dependent effect of the risk allele.

We considered the possibility that intersubject differences in teacher rated hyperactivity might account for the effect in the right posterior orbital gyrus, because this SDQ indicator was associated with NRG1 genotype (Table I). We therefore repeated the statistical analysis including teacher rated hyperactivity as covariates of no interest.

Figure 1.

Brain regions showing greater activation for Go and Nogo trials relative to fixation (top), for Go relative to Nogo trials (middle), and for Nogo relative to Go trials (bottom). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The effect in the right posterior orbital gyrus was replicated when individuals with either one or two risk alleles were contrasted against those without the risk allele ($x = 44$, $y = 24$, $z = -14$, $z\text{-score} = 4.7$, $P = 0.002$ after FWE correction) as well as when individuals with two risk alleles were contrasted against those without it ($x = 44$, $y = 24$, $z = -14$, $z\text{-score} = 4.6$, $P = 0.002$ after FWE correction). This indicates that the effect of NRG1 genotype on brain activation was not attributable to intersubject differences in teacher rated hyperactivity.

To examine whether the effect of NRG1 genotype in the right posterior orbital gyrus was still significant after correction for the nonindependence of twin data, we extracted the individual parameter estimates from this region and then performed a regression analysis based on estimates of the standard errors with and without correction for clustering on family using STATA software (see Methods). When individuals with either one or two risk alleles were contrasted against those without the risk al-

lele, the effect of NRG1 on brain activation was significant both with ($t = 2.91$, $P\text{-value} = 0.005$) and without ($t = 3.23$, $P\text{-value} = 0.001$) correction for independence of twin data. Similarly, when individuals with two risk alleles were contrasted against those without it, the effect of NRG1 was significant both with ($t = 3.08$, $P\text{-value} = 0.003$) and without correction for nonindependence ($t = 4.77$, $P < 0.001$). Estimate of the R^2 measure in STATA revealed that NRG1 genotype accounted for 11% of the interindividual variance in brain activation in this region.

To examine whether the effect of NRG1 genotype in the right posterior orbital gyrus was still significant without the inclusion of twin pairs, we randomly removed one twin from each pair and then repeated the statistical analysis using SPM software (see Methods). When we contrasted individuals with either one or two risk alleles ($n = 36$) against those without the risk allele ($n = 24$), the effect in the right posterior orbital gyrus was replicated ($x = 44$, $y = 24$, $z = -14$, $z\text{-score} = 4.6$, $P = 0.005$ after FWE

TABLE II. Regions expressing increased activation during the Go/Nogo Task relative to fixation, at $P < 0.05$ after FWE correction for multiple comparisons

Go/Nogo task > fixation	Coordinates (x, y, z)	Z score
Left middle frontal gyrus	-36, 50, 28	6.4
Right middle frontal gyrus	38, 50, 32	5.5
Left inferior frontal gyrus	-46, 46, 8	5.1
Right inferior frontal gyrus	38, 54, 8	5.8
Right precentral gyrus	50, 22, 40	6.6
Right insula	50, 16, -4	7.2
Right middle temporal gyrus	62, -40, -2	6.1
Right middle cingulate gyrus	4, -26, 34	5.0
Left angular gyrus	-42, -64, 50	5.2
Right angular gyrus	36, -60, 46	7.2
Left inferior occipital gyrus	-34, -60, -20	7.0
Right inferior occipital gyrus	34, -58, -20	7.2
Left inferior middle occipital gyrus	-42, -82, -10	6.4
Right inferior middle occipital gyrus	38, -84, -12	7.8
Right lingual gyrus	6, -88, -6	7.7
Left lingual gyrus	-4, -88, -6	6.7

TABLE III. Regions expressing different activation for Go and Nogo trials, at $P < 0.05$ after FWE correction for multiple comparisons

	Coordinates (x, y, z)	Z score
Nogo>Go		
Right middle frontal gyrus	42, 22, 42	4.9
	26, -2, 48	4.7
Right precentral gyrus	42, 0, 38	6.6
Right superior temporal gyrus	62, -48, 16	6.3
Right middle temporal gyrus	48, -30, -8	5.2
Right inferior parietal lobule	40, -40, 44	6.6
Right angular gyrus	28, -64, 48	5.7
Right precuneus	8, -62, 50	5.6
Go>Nogo		
Right cerebellum	20, -52, -22	7.2
Left cerebellum	-26, -64, -14	6.9
Right fusiform gyrus	28, -56, -10	5.4
Left fusiform gyrus	-26, -64, -14	6.9
Anterior cingulate gyrus	6, 28, -6	6.6
Left parahippocampal gyrus	-28, -42, -12	5.0
Right parahippocampal gyrus	28, -44, -12	4.7
Left insula	-44, -18, 14	4.9

correction). Likewise, when the three genotypic groups were modeled independently, TT homozygotes expressed greater activation than CC homozygotes in this region ($x = 44, y = 24, z = -14$, z -score = 5.4, $P < 0.001$ after FWE correction).

Interaction Between Stimulus Type and Genotype

The interaction between stimulus type and NRG1 genotype was assessed by testing for effects of genotype on brain function that were significantly different for Go and Nogo trials; this allowed us to identify areas where the effect of genotype differed for response inhibition relative to motor execution. When individuals with one and two risk alleles were combined, we did not detect any significant effects; similarly, no effects were detected when individuals with one and two risk alleles were modeled separately. No "trends" were detected in either analysis even when lowering the statistical threshold to $P < 0.05$ (uncorrected), indicating that the impact of NRG1 on brain function was not dependent on stimulus type (i.e. Go vs Nogo).

DISCUSSION

The aim of this study was to investigate the impact of NRG1, a promising candidate gene for schizophrenia and bipolar disorder, on brain function during the Go/Nogo task in children aged between 10 and 12. We tested the hypothesis that NRG1 would have a measurable impact on brain function in our sample of children.

Previous studies on the impact of NRG1 on brain function have reported that genetic variation in NRG1 affected

brain activation during executive processing in adult participants. Here we expand previous results by reporting that the risk allele is associated with increased activation of the right posterior orbital cortex during performance of the Go/Nogo task in children as young as 10–12 years of age. This is an important observation because reports of an association between the NRG1 genotype and psychotic illness have been based on adult participants; yet the majority of psychopathology does not arise de novo in adults without any warning in childhood or adolescence [Kim-Cohen et al., 2003]. Our finding that genetic variability in

Figure 2.

Left: region of the right posterior orbital gyrus showing a main effect of NRG1 genotype on brain activation. Right: parameter estimates for the contrasts "Go trials > fixation" and "Nogo trials > fixation" in the CC, CT, and TT groups with C and T being the low-risk and high-risk alleles, respectively. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

NRG1 moderates prefrontal processing of 10–12 years of age is consistent with the notion that genetic factors affect brain function to moderate vulnerability to psychopathology from early age [Viding et al., 2006].

In this investigation, variability in NRG1 was associated with differences in orbitofrontal activation for both Nogo than Go stimuli and there is no evidence for a gene $\times$ stimulus type interaction even at trend level. These results could be considered surprising for two main reasons. First, the finding of similar effects of genetic variation for Go and Nogo stimuli may be surprising because response inhibition requires significantly greater allocation of cognitive resources than motor execution [Menon et al., 2001]. However, the two conditions share a number of cognitive processes that could be affected by genetic variation in NRG1; these include visual processing, online monitoring of the stimuli, evaluation of their content and selection of an appropriate response. Our results suggest that genetic variation in NRG1 affects these shared cognitive processes rather than processes specific to either condition such as inhibitory control and motor execution. An alternative interpretation, which cannot be discarded on the basis of the present data, is that genetic variation in NRG1 affects brain function at baseline; for instance, greater activation for Go and Nogo trials relative to fixation could be due to decreased BOLD signal during fixation rather than increased BOLD signal during Go and Nogo trials. Second, the finding of similar effects of genetic variation for Go and Nogo stimuli may be surprising because previous studies of patients with schizophrenia and bipolar disorder had reported functional abnormalities for response inhibition relative to motor execution [Altshuler et al., 2005; Arce et al., 2006; Bark et al., 2005; Ford et al., 2004; Kaladjian et al., 2007; Rubia et al., 2001]. However, we note that none of the previous studies examined functional abnormalities for Go and Nogo stimuli relative to fixation; thus, it is unclear whether patients with schizophrenia and bipolar disorder express functional abnormalities during both response inhibition and executive response relative to fixation. We also note that genetic vulnerability associated with NRG1 is not equivalent to the manifestation of psychotic illness; for instance it is possible that the former is characterized by more general alterations in brain function while the latter is characterized by more specific neurocognitive deficits.

This finding was not confounded by differences in performance or error-related noise in brain activation [Menon et al., 2001], because the three genotypic groups did not differ in terms of response accuracy or reaction times and furthermore incorrect responses were discarded from the statistical analysis by using an event-related model. Thus, the observed difference in the right posterior orbital gyrus indicates that genetic variation in NRG1 is associated with different levels of prefrontal engagement during performance of the Go/Nogo task. We note that the right posterior orbital gyrus was activated in individuals with two high-risk alleles but not in those without the risk allele (see Fig.

2); this pattern of responses suggests that successful task performance was associated with the recruitment of an additional region in individuals with the high-risk genotype relative to those with the low-risk genotype.

The orbitofrontal region identified in this investigation is part of a distributed fronto-limbic network [Hikosaka and Watanabe, 2000] which is implicated in affective processing [Cardinal et al., 2002] as well as decision making [Elliott et al., 2000]. It has been suggested that disrupted functional integration within this network may contribute to dysregulation of motivational, chronobiologic, and endocrinologic processes in psychosis [Blumberg et al., 1999]. Lesions to the orbitofrontal region result in dramatic behavioral changes including impulsivity, poor planning, poor judgement, irritability, and risk-taking behavior [Altshuler et al., 2005]. Interestingly, several neuroimaging studies have reported alterations in structure and function within this network in patients with first episode psychosis as well as individuals at high risk for psychotic illness [Fusar-Poli et al., 2007].

This investigation has a number of limitations. First, we genotyped our subjects for SNP8NRG243177 (rs6994992); this SNP was chosen because (i) it showed a significant association in Steffansson's original Icelandic study [Steffansson et al., 2002]; (ii) it was associated with changes in mRNA expression in a recent investigation [Law et al., 2006]; (iii) it has been shown to have a significant impact on brain structure and function in previous neuroimaging studies on adult participants [Hall et al., 2006; McIntosh et al., 2007]. However, within the NRG1 gene, several other SNPs have been identified by genetic studies and there is no agreement as to which is the most significant marker. An alternative approach might be to use the risk haplotype rather than SNPs to characterize the effect of NRG1 on brain function. However, there are methodological problems with this strategy, because haplotypes are not absolute measures in phase-unknown populations but are assigned by probability using a complex likelihood calculation [Curtis and Xu, 2007; Moskvina and Schmidt, 2006]. A second limitation is that our sample only included males and therefore it is unclear whether the results can be generalized to the female population; given that psychotic disorders have been reported to show gender differences in their nature and incidence, it is important that future studies should examine the extent to which the impact of candidate genes on brain physiology is modulated by gender. A third limitation is that our sample included typically developing children as well as a small fraction of children in the top 10% of the U.K. population for conduct problems (see Methods). However, none of the children had an official conduct disorder diagnosis and the mean score on conduct problems was not in the abnormal range. A fourth limitation is that our sample also included several pairs of twins which may result in biased estimate of standard errors (see Methods); nevertheless, our finding was still significant when statistical inferences were made after correction for nonindependence of

twin data and when the analysis was repeated after randomly removing one twin from each pair. A fifth limitation is that the superior prefrontal and parietal cortices were not included in our statistical analysis because these regions were not scanned in a subset of subjects; it is therefore unclear whether brain activation in these regions was modulated by NRG1 genotype during the Go/Nogo task. A sixth limitation is that our statistical analysis did not distinguish between Go trials presented within blocks which did not include any Nogo stimuli, and Go trials presented within blocks which included Nogo stimuli; the two sets of Go trials might be associated with different neurocognitive processes. In particular, the former might elicit a greater degree of cognitive and neuronal habituation than the latter. A final limitation is that a more comprehensive understanding of how variation in NRG1 genotype affects brain function to increase susceptibility to mental illness will require investigation of interactions with other candidate genes and with environmental risk factors [Caspi and Moffitt, 2006].

In conclusion, this is the first investigation to examine the effects of genetic variation in NRG1 on brain responses in children. We report that the high and low risk genotypes are associated with different levels of prefrontal engagement during both response inhibition and motor execution in children as young as 10–12 years of age. Our investigation provides support to the idea that genetic variation in NRG1 affects brain function to moderate vulnerability to psychopathology from childhood, before the manifestation of any symptoms in late adolescence and adulthood.

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