Behav Genet (2010) 40:157–166
DOI 10.1007/s10519-009-9317-5

ORIGINAL RESEARCH

Influence of Neuregulin1 Genotype on Neural Substrate of Perceptual Matching in Children

Andrea Mechelli · Essi Viding · Atul Kumar · Stefania Tognin · Fergus Kane · Philip McGuire

Received: 29 January 2009 / Accepted: 18 November 2009 / Published online: 5 December 2009

Abstract Adult psychopathology is often rooted early in life and first emerges during childhood and adolescence. However, as most imaging genetic research to date has involved adult participants, little is known about how risk genes affect brain function to influence biological vulnerability in childhood. We examined the impact of neuregulin1 (NRG1), a probable susceptibility gene for schizophrenia and bipolar disorder, on brain function in a sample of 102 healthy 10–12 year old boys including 18 pairs of monozygotic twins, 24 pairs of dizygotic twins and 18 singletons. Each participant performed a perceptual matching task, while brain responses were measured using functional magnetic resonance imaging. Response accuracy and reaction times did not differ as a function of NRG1 genotype; however, individuals with two high-risk alleles showed relatively increased brain activation in a distributed network comprising the precuneus bilaterally, and the left cuneus, middle occipital gyrus, angular gyrus and caudate nucleus. These results indicate that genetic variation in NRG1 significantly affects cortical function during perceptual and monitoring processes in healthy children as young as 10–12 years of age.

Keywords Neuregulin1 · Schizophrenia · Bipolar disorder · Childhood · Perceptual matching · Functional magnetic resonance imaging

Edited by William Kremen.

A. Mechelli (✉)
Department of Psychology, Institute of Psychiatry, King's College London, 103 Denmark Hill, PO BOX 67, London SE5 8AF, UK
e-mail: a.mechelli@kcl.ac.uk

A. Mechelli · A. Kumar · S. Tognin · F. Kane · P. McGuire
Section of Neuroimaging, Division of Psychological Medicine and Psychiatry, Institute of Psychiatry, King's College London, 103 Denmark Hill, London SE5 8AF, UK

E. Viding
Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, 103 Denmark Hill, London SE5 8AF, UK

E. Viding
Division of Psychology and Language Sciences, University College London, Gower St., London WC1E 6BT, UK

Introduction

Schizophrenia and bipolar disorder are severe psychiatric disorders that develop in adolescence and early adulthood and have 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), it is important to extend the current imaging genetic work to include child samples. We therefore 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 a perceptual matching task in 10–12 year old boys.

Neuregulin1, located at 8p21–p22, was originally associated with schizophrenia by Stefansson et al. (2003, 2002), who found association of a so-called "core haplotype" in the NRG1 gene with a two-fold 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 (Thomson et al. 2007; Green et al. 2005) have implicated NRG1 in bipolar disorder. It should be noted that not all studies have detected a significant association (see Ikeda et al. 2008 for review) and that there have been inconsistencies in terms of the regions of the gene associated with illness. However, taken collectively, the studies published so far suggest that NRG1 may confer biological susceptibility to psychopathology across the traditional Kraepelian dichotomy of affective and non-affective 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, which are known to participate in ErbB signaling (Steinthorsdottir et al. 2004; Falls 2003). 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 psychiatric 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 by contrasting the allele associated with a higher frequency of cases (T; "risk allele") against that associated with a smaller frequency of cases (C). Hall et al. (2006) found that relatives of people with schizophrenia with the risk allele of the NRG1 gene showed reduced medial prefrontal and temporo-occipital activation during a sentence completion task. 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. (2009) found reduced activation of the hippocampus, precuneus, cerebellum and anterior cingulate in first episode schizophrenic patients with the risk variant performing a working memory tasks. Finally, Mechelli et al. (2008) demonstrated differential effects of NRG1 genotype on prefrontal function in healthy volunteers and patients with schizophrenia and bipolar disorder during performance of a verbal fluency task. In all 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 cognitive processing and furthermore provide evidence for a disease-specific pattern of gene action in different regions of the prefrontal cortex. Interestingly, NRG1-related differences in brain activation were not associated with differences in behavioural performance in any of the studies; this is consistent with the idea that the direct assay of brain physiology provided by neuroimaging techniques can be more sensitive to the functional impact of genetic variation than standard behavioural measures (Hariri and Weinberger 2003). However, it should be noted that different studies focused on different single nucleotide polymorphisms; in particular Hall et al. (2006) examined rs6994992 while Krug et al. (2008), Kircher et al. (2009) and Mechelli et al. (2008) examined rs35753505.

It is currently unclear whether NRG1 genotype influences brain responses in children. This is an important question since schizophrenia and bipolar disorder are thought to be neurodevelopmental disorders, and the associated risk genes are believed to act through disruption of normal brain development (Weinberger 1987). If gene-related differences in adult participants reflect alterations that occurred during childhood and adolescence, then a better characterization of these alterations in children and adolescents is critical for understanding how genes affect brain 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 gray matter volume in fronto-temporal regions is already evident during adolescence and significantly differs between patients with childhood onset schizophrenia and healthy controls (Addington et al. 2007). The aim of the present study was to examine the effects of NRG1 genotype on brain function in children.

We used functional Magnetic Resonance Imaging (fMRI) to explore the impact of the rs35753505 single nucleotide polymorphism of the NRG1 genotype in 102 children aged between 10 and 12, using a perceptual matching paradigm which required participants to match an image with one of two other images. This simple task could be easily performed by children in a scanning environment, and engages perceptual and monitoring processes, which are known to be adversely affected in patients with psychotic disorders (Butler and Javitt 2005; Krabbendam et al. 2005). Based on the results of previous studies with adult

participants who did not have schizophrenia diagnosis (Krug et al. 2008; Mechelli et al. 2008), we hypothesized that children with the risk variant of the gene would express greater activation than those without it, indicating that NRG1 affects cortical function not only in adults, but also in children. Although we examined the whole brain and corrected for multiple comparisons accordingly, we expected NRG1-related differences to be expressed in regions that are normally crucial for perceptual and monitoring processes, such as the occipital and parietal cortices. As the data were originally acquired as part of an on-going twin neuroimaging project which included boys with conduct problems as well as typically developing boys, the emotional content of the stimuli was also manipulated to include both emotional and neutral images. While we examined the impact of this manipulation on NRG1-related differences for completeness, we did not expect this to be significant, given the lack of evidence for a specific role of NRG1 in emotional processing. For instance, a recent investigation reported that 'knockout' of the gene in the adult mice disrupts social dyadic interactions but not emotional/anxiety-related behaviour (O'Tuathaigh et al. 2008). Finally, we did not expect NRG1-related differences in brain activation to be associated with differences in behavioural performance, consistent with the results of previous investigations with adult participants (Hall et al. 2006; Krug et al. 2008; Kircher et al. 2009; Mechelli et al. 2008).

Methods

Subjects

A total of 102 10–12 years old boys participated in the present study. All 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 UK 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 not in the abnormal range, nor did our sample have abnormal 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 1). None of the children, including those typically developing as well as those in the top 10% of the UK 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; in order to account for the non-independence 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

Table 1 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
Task performance					
Errors (%)	3.91 (5.44)	3.24 (3.12)	2.08 (1.20)	3.44 (4.18)	0.16
RT (ms)	1435 (256)	1377 (245)	1487 (297)	1409 (253)	0.39

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

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 (1) it showed a significant association in Steffansson's original Icelandic study (Stefansson et al. 2002); (2) it was associated with changes in mRNA expression in a recent investigation (Law et al. 2006); (3) 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 (Goodman et al. 2000) was used to measure conduct problems, hyperactivity, emotional problems (a screen for depression and anxiety), peer problems and prosociality in all participants. The three genotypic groups were compared using a regression analysis which accounted for the non-independence of twin data, using STATA software (version 10). In particular we used the clustered robust (also called Huber-White) standard error method in order to estimate standard errors which were corrected for clustering on family (Diggle et al. 2002). Demographic, cognitive and behavioural data are described in the Results section and summarized for each genotype group in Table 1.

Genotyping

Genomic DNA was isolated from buccal swab samples (Freeman et al. 2003). Genotyping of the single nucleotide polymorphism SNP8NRG243177 (rs6994992) was performed on the 3130 Genetic Analyzer and using the ABI PRISM® SNaPshot™ Multiplex Kit (Applied Biosystems, Foster City, CA, USA). We included a negative control to check for cross-contamination. The genotyping of a sample of 121 subjects, which included our 102 participants, yielded 2 failures which could not be repeated due to limits of source DNA. Our observed genotypes were found to be within Hardy–Weinberg equilibrium.

Perceptual matching task

Each stimulus comprised three images, with one presented at the bottom of the screen and two presented in the top left and right corners, respectively. Subjects were instructed to click a left or right button on a key pad, depending on whether the image at the bottom matched the image presented in the top left or top right of the screen. The images within each stimulus were either colour photographs with high negative emotional content (e.g., spiders, snarling dogs) taken from the International Affective Picture System (Lang et al. 2005; see "Appendix" for full list), or geometric shapes including vertical and horizontal ellipses. A total of 48 stimuli (24 photographs with high emotional content, 24 geometric shapes) were presented, with each stimulus remaining on the screen for 3 s and an interval between stimuli of 2 s, resulting in a stimulus onset asynchrony of 5 s; this constituted the allotted time for the subject to complete each individual matching trial. Two blocks of rest, each lasting 34 s, were also included throughout the experiment; during these blocks of rest participants were instructed to focus on a fixation cross in the middle of the screen. Thus, total acquisition time was 308 s.

Image acquisition

Neuroimaging data were acquired using a General Electric Signa 3.0 Telsa Excite II MRI scanner (Medical systems, Milwaukee, WI, USA) at the Centre for Neuroimaging Science, Institute of Psychiatry. In order to exclude the presence of gross anatomical abnormalities, a structural brain image was acquired from each subject using an isotropic resolution 3D inversion recovery prepared spoiled gradient echo (IR SPGR). Parameters for the IR-SPGR were TR = 8 ms; TE = 2.9 ms; TI = 450 ms; excitation FA = 20°. The in-plane matrix size was 256 × 192 over a 280 × 210 mm field of view, reconstructed to 256 × 256 over 280 × 280 mm. Two hundred through plane partitions (each 1.1 mm thick) were collected, with two partitions being discarded at each end of the imaging volume to minimize wrap-round artefacts, resulting in a scanning time of 6 min. In addition, a total of 160 functional images, each comprising 28 slices, were acquired in a single run with a T2*-weighted gradient echo-planar imaging sequence (EPI) and a repetition time (RT) of 2 s (slice thickness 3.5 mm, gap = 0.3 mm; TE = 25 ms, field of view = 220 × 220, matrix size 64 × 64). The orientation of the axial slices was parallel to the ACPC line. Stimuli were projected onto a high-resolution screen located in front of the participant's head, and were viewed via mirror attached to the head coil. They were presented using Visual Basic software and synchronized with pulses generated by the scanner at the beginning of each scan sequence.

Image 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 functional magnetic resonance imaging (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., Marsh et al. 2008; Bitan et al. 2009; Jones 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 haemodynamic response function (HRF). In order to minimize performance confounds, incorrect trials in which the subject did not respond correctly were discarded using an event-related model (Mechelli et al. 2003). In order 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 calculating the parameter estimates for all voxels using the general linear model, a contrast image was computed for the comparison between visual stimuli and fixation in each subject independently. Second, the subject-specific contrast images were entered into an ANOVA to identify effects of genotype expressed consistently across subjects (Penny and Holmes 2003). Statistical inferences were made using a statistical threshold of $p < 0.05$ with family-wise error (FWE) correction for multiple comparisons across the whole brain and an extent threshold of 10 voxels.

Correction for non-independence of twin data

Because SPM software does not allow correction for the non-independence of twin data, we extracted the individual parameter estimates from the peak voxel of regions expressing significant effects of NRG1 genotype in the SPM analysis, and then used STATA software (version 10) to compare the different genotypic groups using the clustered robust (also called Huber-White) standard error method (Diggle et al. 2002). This procedure allowed us to examine whether the effects of NRG1 genotype identified with SPM were still significant after correction for the non-independence of twin data based on estimates of the standard errors corrected for clusterig on family. 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 inter-individual variance in BOLD activation was explained by the genetic variation.

Results

Demographics

Table 1 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 or 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 ($p < 0.05$).

Behavioural 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 non-independence of twin data. NRG1 genotype was not associated with response accuracy ($F = 1.88$; df = 2,58; $p = 0.16$) or reaction times ($F = 0.94$; df = 2,58; $p = 0.39$). Table 1 reports the number of errors and the reaction times for each genotypic group and for all subjects combined.

Effect of perceptual matching versus fixation

A widely distributed bilateral network comprising frontal, temporal, occipital, parietal and subcortical areas expressed increased activation during perceptual matching relative to fixation (Fig. 1).

Effect of NRG1 genotype

The effect of NRG1 genotype was examined by comparing brain activation during perceptual matching relative to

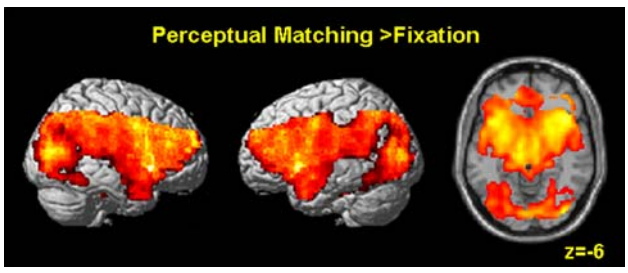

Fig. 1 Brain regions showing greater activation during the perceptual matching task relative to fixation ($p < 0.05$ after FWE correction across the whole brain)

fixation between different genotype groups. We report the results for emotional and neutral images combined since, in line with our prediction, an initial statistical analysis did not reveal an interaction between NRG1 genotype and the emotional content of the images (even with a statistical threshold as relaxed as $p < 0.05$ uncorrected). There were no regions showing *reduced* activation in TT homozygotes relative to either CC homozygotes and CT heterozygotes. However, TT homozygotes expressed *greater* activation relative to both CC homozygotes and CT heterozygotes in a predominantly left-sided distributed network comprising the left caudate nucleus, precuneus, cuneus, middle occipital gyrus and angular gyrus, plus the right precuneus (see Table 2; Fig. 2). In all these regions, individuals with the TT genotype showed strong activation during perceptual matching relative to fixation ($p < 0.05$ after FWE correction), whereas individuals with the CC or the CT genotype showed minimal or no activation.

We considered the possibility that the impact of NRG1 genotype on brain function might be explained by inter-subject differences in teacher rated hyperactivity, since this SDQ indicator was also associated with NRG1 genotype (Table 1). We therefore repeated the statistical analysis including teacher rated hyperactivity as a covariate of no interest. All the significant effects identified above were replicated (at $p < 0.05$ after FWE correction), suggesting that the impact of NRG1 genotype on brain activation was not attributable to associated differences in ratings of hyperactivity.

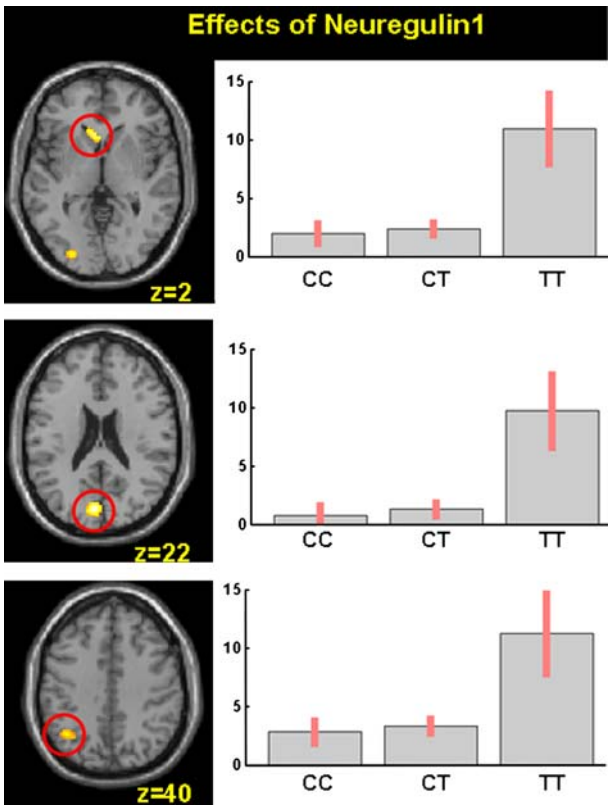

Fig. 2 Effects of NRG1 genotype on brain function ($p < 0.05$ after FWE correction across the whole brain) in the left caudate nucleus (*top*), left precuneus (*middle*) and left angular gyrus (*bottom*). Parameter estimates are shown for each genotypic group where T and C are the high-risk and low-risk alleles, respectively

Table 2 Effects of NRG1 genotype on brain function

Region	Coordinates (*x y z*)	SPM		STATA		
		TT > CC	TT > CT	TT > CC	TT > CT	R^2
Left caudate nucleus	−6 22 2	5.6	5.6	$t = 2.04$ ($t = 4.02$) $p = 0.046$ ($p < 0.001$)	$t = 1.80$ ($t = 3.46$) $p = 0.077$ ($p = 0.001$)	17% (14%)
Left precuneus	−4 −82 24	5.4	5.5	$t = 5.20$ ($t = 3.85$) $p < 0.001$ ($p < 0.001$)	$t = 4.16$ ($t = 3.43$) $p < 0.001$ ($p = 0.001$)	14% (13%)
Right precuneus	16 −82 16	5.4	5.4	$t = 4.83$ ($t = 3.72$) $p < 0.001$ ($p < 0.001$)	$t = 3.90$ ($t = 3.39$) $p < 0.001$ ($p = 0.001$)	14% (12%)
Left cuneus	−2 −90 14	4.9	5.6	$t = 2.13$ ($t = 3.23$) $p < 0.038$ ($p = 0.002$)	$t = 2.27$ ($t = 3.35$) $p < 0.027$ ($p = 0.001$)	12% (10%)
Left middle occipital	−26 −88 2	4.8	4.6	$t = 2.43$ ($t = 3.57$) $p < 0.018$ ($p = 0.001$)	$t = 1.93$ ($t = 2.87$) $p = 0.054$ ($p = 0.005$)	13% (11%)
Left angular gyrus	−38 −58 38	4.5	4.7	$t = 3.53$ ($t = 3.26$) $p = 0.001$ ($p = 0.002$)	$t = 2.83$ ($t = 2.61$) $p = 0.006$ ($p = 0.011$)	11% (9%)

In the SPM columns, *z*-scores are reported for the comparisons TT > CC (i.e., TT homozygotes > CC homozygotes) and TT > CT (i.e., TT homozygotes > CT heterozygotes); all effects were significant at $p < 0.05$ after correction for multiple comparisons across the whole brain. In the STATA columns, *t* scores and *p*-values are reported with correction for non-independence of twin data as well as without such correction (in brackets). R^2 provides an estimate of how much of the inter-individual variance in BOLD activation is explained by the genetic variation and is reported with correction for non-independence of twin data as well as without such correction (in brackets)

In order to examine the impact of NRG1 genotype on brain function after correction for the non-independence of twin data, we extracted the individual parameter estimates from regions showing a significant effect, then performed a series of regression analyses based on estimates of the standard errors with and without correction for clustering on family, using STATA software. Correction for the non-independence of twin data resulted in increased statistical significance (i.e., increased t scores and decreased p values) in the left and right precuneus and the left angular gyrus, and decreased statistical significance (i.e., decreased t scores and increased p values) in the left caudate, cuneus and middle occipital gyrus (Table 2). This indicated that despite slight changes in the degree of significance, the effects of NRG1 on regional brain activation were replicated (at $p < 0.05$) after correction for the non-independence of twin data. The only exception was in the left caudate nucleus, where the effect on activation was no longer significant for the comparison TT homozygotes > CT heterozygotes following correction (Table 2). Estimate of the R^2 measure in STATA revealed that NRG1 genotype accounted for 11–17 and 9–14% of the inter-individual variance with and without correction for the non-independence of twin data, respectively (see Table 2).

Discussion

The aim of the present study was to investigate the impact of NRG1, a candidate gene for schizophrenia and bipolar disorder, on brain function during a perceptual matching task in healthy children. We tested the hypothesis that NRG1 would have a measurable impact on regional activation in children, consistent with evidence that psychopathology in adults is often predated by signs of altered function or behaviour in childhood or adolescence (Kim-Cohen et al. 2003). More specifically, we predicted that individuals with the high-risk variant of the gene would express greater activation in regions crucial for perceptual and monitoring processes, particularly the occipital and parietal cortices, based on previous studies with healthy adult participants (Krug et al. 2008; Mechelli et al. 2008). While we did not expect the impact of the NRG1 gene to differ between emotional and neutral stimuli, we also tested for an interaction between this gene and the emotional content of the images for completeness.

Consistent with our main prediction, we found that children with two high-risk alleles expressed increased activation during perceptual matching relative to fixation in a distributed network involved in perceptual and monitoring processes, comprising the precuneus bilaterally and the left caudate nucleus, cuneus, middle occipital and angular gyrus. In these regions, NRG1 genotype accounted for between 11 and 17% of the inter-individual variance in brain activation (Table 2). However, NRG1 genotype had no impact on activation during the processing of emotional as opposed to neutral stimuli, even when lowering the statistical threshold to $p < 0.05$ (uncorrected), consistent with the notion that this gene does not play specific role in emotional processing.

The effects of genotype we observed are unlikely to be driven by differences in performance, since 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 employing an event-related model. The observation of NRG1-related differences in brain function in the absence of differences is consistent with the results of previous studies with adult participants (Hall et al. 2006; Krug et al. 2008; Mechelli et al. 2008; Kircher et al. 2009). In addition, the findings are unlikely to be explained by NRG1-related differences in teacher rated hyperactivity, as inclusion of this variable as a covariate of no interest did not change the results. The most likely explanation for these effects is therefore that genetic variability in NRG1 moderates cortical function in healthy children as young as 10–12 years of age. We note that those regions which were significantly modulated by NRG1 activated in individuals with two high risk alleles but not in those without the risk allele (Fig. 2); this pattern of responses suggests that successful task performance was associated with the recruitment of additional regions in individuals at higher risk relative to those at lower risk. This is an important observation, since hitherto, reports of an association between the NRG1 genotype and psychotic illness have been based on data from adult participants. However, schizophrenia is a neurodevelopmental disorder and risk genes are thought to act through a disruption of normal brain development (Weinberger 1987). Our findings are consistent with the notion that genetic factors affect brain function to moderate vulnerability to psychopathology from early age, long before the expression of clinical symptoms in late adolescence and adulthood.

Interestingly, we found that the effects of NRG1 on cortical function were driven by strong activation in individuals with two high-risk alleles, while individuals with one or no high-risk allele expressed minimal or no activation; this pattern of response parallels the results of previous neuroimaging studies in healthy adults (Hall et al. 2006; Krug et al. 2008). We speculate that increased activation in individuals with two high-risk alleles might reflect more effortful processing during task performance, although this raises the question of why the opposite pattern (i.e., decreased activation in individuals with the high-risk alleles) has been found in previous studies of participants with schizophrenia (Kircher et al. 2009; Mechelli et al. 2008).

Neuregulin1 affected brain responses during perceptual matching relative to fixation in regions of the visual cortex, including the left cuneus and the left middle occipital gyrus. Alterations in visual processing have been consistently demonstrated in patients with schizophrenia using behavioural tests (e.g., Schechter et al. 2003), as well as a range of neuroimaging techniques (Butler et al. 2005; Martínez et al. 2008), These alterations are particularly evident in the magnocellular system, which is glutamate/NMDA-dependent, but can also be observed in the parvocellular system (Butler and Javitt 2005). Based on the implication of NRG1 in glutamate/NMDA neurotransmission, we speculate that the effects which we observed in the occipital cortex might contribute to some of the alterations in the magnocellular system of patients with schizophrenia.

Neuregulin1 also influenced cortical function in two parietal regions which have strong anatomical connections, namely the angular gyrus and the precuneus (Cavanna and Trimble 2006). The angular gyrus is structurally (Nierenberg et al. 2005) and functionally (Pauly et al. 2008) altered in schizophrenia, and is most likely to mediate cognitive aspects of our perceptual matching task, such as monitoring processes. Similarly, the precuneus is implicated in a wide spectrum of highly integrated tasks, including visuo-spatial imagery, episodic memory retrieval and self-processing operations (see Cavanna and Trimble for review), and has been reported to be over-active in patients with schizophrenia during decision making (Paulus et al. 2002).

Finally, an effect of NRG1 was detected in the head of the caudate nucleus, which plays a major part in high cognitive function and movement control (Middleton and Strick 1994) and is structurally and functionally altered in individuals with schizophrenia as well as their unaffected relatives (Rajarethinam et al. 2007; Raemaekers et al. 2006; Keshavan et al. 2005). The observation of increased brain activation of this region in individuals with two high-risk alleles provides support to the notion that an alteration of the structural and functional integrity of the basal ganglia nuclei may represent familial or premorbid risk of schizophrenia (Keshavan et al. 2005). Nevertheless this finding should be interpreted with caution and required replication in a non-twin sample, as it was no longer significant following correction for the non-independence of the data (Table 2).

The present investigation has a number of potential limitations. First, we genotyped our subjects for SNP8-NRG243177 (rs6994992); this SNP was chosen because (1) it showed a significant association in Steffansson's original Icelandic study (Stefansson et al. 2002); (2) it was associated with changes in mRNA expression in a recent investigation (Law et al. 2006); (3) 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 in order to characterize the effect of NRG1 on brain function. However, there are methodological problems with this strategy, since 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 it is therefore unclear whether the results can be generalized to the female population. Given that psychotic disorders show gender differences in their incidence, age of onset and course, it is important that future studies should examine the extent to which the impact of candidate genes on brain physiology is modulated by gender. On the other hand, restricting the sample to males is likely to have resulted in increased statistical power by reducing any gender related heterogeneity. 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 UK population for conduct problems (see "Methods"). However, none of the children had an official conduct disorder diagnosis and the mean score on conduct problems for our sample was not in the abnormal range. More critically, the genotype groups did not differ in their levels of conduct problems. A fourth limitation is that our sample included several pairs of twins which may result in biased estimate of standard errors. STATA package allows correction for non-independence of twin data but is not specifically designed for neuroimaging data and therefore cannot be used to analyze thousands of voxels at once and take spatial dependency into account; conversely SPM software allows all voxels to be considered but cannot be used to correct for non-independence of twin data. We therefore (1) performed the analysis in SPM, (2) extracted the signal from all significant regions and (3) ran them through the STATA package. Despite slight changes in the degree of significance, our findings were replicated, and some effects became even more significant, when statistical inferences were made after correction for non-independence of twin data. 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). Future studies should also examine the relationship between NRG1 genotype and teacher rated hyperactivity to assess whether this is a true finding or a spurious association; such association was not predicted on the basis of previous studies and as such should requires replication.

In conclusion, this is the first investigation to examine the effects of genetic variation in NRG1 on brain responses during perceptual matching in children. We report that the high-risk variant is associated with increased brain activation during a task engaging perceptual and monitoring processes in children as young as 10–12 years of age, consistent with previous findings with healthy adult participants. Our results are consistent with the idea that genetic variation in NRG1 affects cortical function to moderate vulnerability to psychopathology from childhood, before the possible manifestation of any symptoms in late adolescence and adulthood. In order to establish whether the genotypic effects reported in the present investigation are related to clinical symptomatology later in life, a longitudinal approach will be required.

Appendix

Identification numbers of the IAPS images (Lang et al. 2005) used in the perceptual matching task

Trial	Image 1	Image 2	Image 3
1	1050	1120	1050
2	9050	9611	9050
3	1220	1240	1240
4	9910	9920	9910
5	1300	1302	1302
6	9622	6900	6900
7	5940	5920	5940
8	1300	1302	1302
9	1930	1931	1931
10	9630	9622	9630
11	8480	9230	9230
12	9600	9620	9600
13	9910	9920	9910
14	1300	1302	1302
15	9622	6900	6900
16	1050	1120	1050
17	9050	9611	9050
18	1220	1240	1240
19	9630	9622	9630
20	8480	9230	9230
21	9600	9620	9600
22	5940	5920	5940
23	9910	9920	9910
24	1930	1931	1931

Each stimulus comprised three images, with one presented at the bottom of the screen (image 3) and two presented in the top left (image 1) and right (image 2) corners, respectively

References

Addington AM, Gornick MC, Shaw P, Seal J, Gogtay N, Greenstein D et al (2007) Neuregulin 1 (8p12) and childhood-onset schizophrenia: susceptibility haplotypes for diagnosis and brain developmental trajectories. Mol Psychiatry 12(2):195–205

Bitan T, Cheon J, Lu D, Burman DD, Booth JR (2009) Developmental increase in top-down and bottom-up processing in a phonological task: an effective connectivity, fMRI study. J Cogn Neurosci 21(6):1135–1145

Burgund ED, Kang HC, Kelly JE, Buckner RL, Snyder AZ, Petersen SE et al (2002) The feasibility of a common stereotactic space for children and adults in fMRI studies of development. Neuroimage 17(1):184–200

Butler PD, Javitt DC (2005) Early-stage visual processing deficits in schizophrenia. Curr Opin Psychiatry 18(2):151–157

Butler PD, Zemon V, Schechter I, Saperstein AM, Hoptman MJ, Lim KO et al (2005) Early-stage visual processing and cortical amplification deficits in schizophrenia. Arch Gen Psychiatry 62(5):495–504

Caspi A, Moffitt TE (2006) Gene-environment interactions in psychiatry: joining forces with neuroscience. Nat Rev Neurosci 7(7):583–590 Review

Cavanna AE, Trimble MR (2006) The precuneus: a review of its functional anatomy and behavioural correlates. Brain 129(Pt 3):564–583 Review

Corfas G, Roy K, Buxbaum JD (2004) Neuregulin 1-erbB signaling and the molecular/cellular basis of schizophrenia. Nat Neurosci 7:575–580

Craddock N, O'Donovan MC, Owen MJ (2007) Phenotypic and genetic complexity of psychosis. Invited commentary on Schizophrenia: a common disease caused by multiple rare alleles. Br J Psychiatry 190:200–203

Curtis D, Xu K (2007) Minor differences in haplotype frequency estimates can produce very large differences in heterogeneity test statistics. BMC Genet 8:38

Diggle PJ, Heagerty P, Liang K-Y, Zeger SL (2002) Analysis of longitudinal data, 2nd edn. Oxford University Press, Oxford, pp 70–80

Falls DL (2003) Neuregulins: functions, forms, and signaling strategies. Exp Cell Res 284(1):14–30 Review

Fisher PM, Muñoz KE, Hariri AR (2008) Identification of neurogenetic pathways of risk for psychopathology. Am J Med Genet C Semin Med Genet 148(2):147–153 Review

Freeman B, Smith N, Curtis C, Huckett L, Mill J, Craig IW (2003) DNA from buccal swabs recruited by mail: evaluation of storage effects on long-term stability and suitability for multiplex polymerase chain reaction genotyping. Behav Genet 33(1):67–72

Friston KJ (2003) Introduction: experimental design and statistical parametric mapping. In: Frackowiak RSJ, Friston KJ, Frith C, Dolan R, Friston KJ, Price CJ, Zeki S, Ashburner J, Penny WD (eds) Human brain function. Academic Press, London, pp 599–632

Goodman R, Ford T, Simmons H, Gatward R, Meltzer H (2000) Using the strengths and difficulties questionnaire (SDQ) to screen for child psychiatric disorders in a community sample. Br J Psychiatry 177:534–539

Green EK, Raybould R, Macgregor S, Gordon-Smith K, Heron J, Hyde S et al (2005) Operation of the schizophrenia susceptibility gene, neuregulin 1, across traditional diagnostic boundaries to increase risk for bipolar disorder. Arch Gen Psychiatry 62(6):642–648

Hall J, Whalley HC, Job DE, Baig BJ, McIntosh AM, Evans KL et al (2006) A neuregulin 1 variant associated with abnormal cortical function and psychotic symptoms. Nat Neurosci 9:1477–1478

Hariri AR, Weinberger DR (2003) Imaging genomics. Br Med Bull 65:259–270

Ikeda M, Takahashi N, Saito S, Aleksic B, Watanabe Y, Nunokawa A et al (2008) Failure to replicate the association between NRG1 and schizophrenia using Japanese large sample. Schizophr Res 101(1–3):1–8

Jones AP, Laurens KR, Herba CM, Barker GJ, Viding E (2009) Amygdala hypoactivity to fearful faces in boys with conduct problems and callous-unemotional traits. Am J Psychiatry 166(1):95–102

Kang HC, Burgund ED, Lugar HM, Petersen SE, Schlaggar BL (2003) Comparison of functional activation foci in children and adults using a common stereotactic space. Neuroimage 19(1): 16–28

Keshavan MS, Diwadkar VA, Montrose DM, Rajarethinam R, Sweeney JA (2005) Premorbid indicators and risk for schizophrenia: a selective review and update. Schizophr Res 79(1):45–57 Review

Kim-Cohen J, Caspi A, Moffitt TE, Harrington H, Milne BJ, Poulton R (2003) Prior juvenile diagnoses in adults with mental disorder: developmental follow-back of a prospective-longitudinal cohort. Arch Gen Psychiatry 60(7):709–717

Kircher T, Thienel R, Wagner M, Reske M, Habel U, Kellermann T et al (2009) Neuregulin 1 ICE-single nucleotide polymorphism in first episode schizophrenia correlates with cerebral activation in fronto-temporal areas. Eur Arch Psychiatry Clin Neurosci 259(2):72–79

Krabbendam L, Arts B, van Os J, Aleman A (2005) Cognitive functioning in patients with schizophrenia and bipolar disorder: a quantitative review. Schizophr Res 80(2–3):137–149

Krug A, Markov V, Eggermann T, Krach S, Zerres K, Stöcker T et al (2008) Genetic variation in the schizophrenia-risk gene neuregulin1 correlates with differences in frontal brain activation in a working memory task in healthy individuals. Neuroimage 42(4):1569–1576

Lang PJ, Bradley MM, Cuthbert BN (2005) International affective picture system (IAPS): affective ratings of pictures and instruction manual (tech report A-6). University of Florida, Center for Research in Psychophysiology, Gainesville

Law AJ, Lipska BK, Weickert CS, Hyde TM, Straub RE, Hashimoto R et al (2006) Neuregulin 1 transcripts are differentially expressed in schizophrenia and regulated by 5′ SNPs associated with the disease. Proc Natl Acad Sci USA 103(17):6747–6752

Li D, Collier DA, He L (2006) Meta-analysis shows strong positive association of the neuregulin 1 (NRG1) gene in schizophrenia. Human Mol Genet 15(12):1995–2002

Li B, Woo RS, Mei L, Malinow R (2007) The neuregulin-1 receptor erbB4 controls glutamatergic synapse maturation and plasticity. Neuron 54(4):583–597

Marsh AA, Finger EC, Mitchell DG, Reid ME, Sims C, Kosson DS et al (2008) Reduced amygdala response to fearful expressions in children and adolescents with callous-unemotional traits and disruptive behavior disorders. Am J Psychiatry 165(6):712–720

Martínez A, Hillyard SA, Dias EC, Hagler DJ Jr, Butler PD, Guilfoyle DN et al (2008) Magnocellular pathway impairment in schizophrenia: evidence from functional magnetic resonance imaging. J Neurosci 28(30):7492–7500

McIntosh AM, Moorhead TW, Job D, Lymer GK, Muñoz Maniega S, McKirdy J et al (2007) The effects of a neuregulin 1 variant on white matter density and integrity. Mol Psychiatry 13(11):1054–1059

Mechelli A, Henson RN, Price CJ, Friston KJ (2003) Comparing event-related and epoch analysis in blocked design fMRI. Neuroimage 18(3):806–810

Mechelli A, Prata DP, Fu CH, Picchioni M, Kane F, Kalidindi S et al (2008) The effects of neuregulin1 on brain function in controls and patients with schizophrenia and bipolar disorder. Neuroimage 42(2):817–826

Middleton FA, Strick PL (1994) Anatomical evidence for cerebellar and basal ganglia involvement in higher cognitive function. Science 266:458–461

Moskvina V, Schmidt KM (2006) Susceptibility of biallelic haplotype and genotype frequencies to genotyping error. Biometrics 62(4):1116–1123

Nierenberg J, Salisbury DF, Levitt JJ, David EA, McCarley RW, Shenton ME (2005) Reduced left angular gyrus volume in first-episode schizophrenia. Am J Psychiatry 162(8):1539–1541

O'Tuathaigh CM, O'Connor AM, O'Sullivan GJ, Lai D, Harvey R, Croke DT, Waddington JL (2008) Disruption to social dyadic interactions but not emotional/anxiety-related behaviour in mice with heterozygous 'knockout' of the schizophrenia risk gene neuregulin-1. Prog Neuropsychopharmacol Biol Psychiatry 32(2):462–466

Paulus MP, Hozack NE, Zauscher BE, Frank L, Brown GG, McDowell J, Braff DL (2002) Parietal dysfunction is associated with increased outcome-related decision-making in schizophrenia patients. Biol Psychiatry 51(12):995–1004

Pauly K, Seiferth NY, Kellermann T, Backes V, Vloet TD, Shah NJ et al (2008) Cerebral dysfunctions of emotion-cognition interactions in adolescent-onset schizophrenia. J Am Acad Child Adolesc Psychiatry 47(11):1299–1310

Penny WD, Holmes A (2003) Random effects analysis. In: Frackowiak RSJ, Friston KJ, Frith C, Dolan R, Friston KJ, Price CJ, Zeki S, Ashburner J, Penny WD (eds) Human brain function. Academic Press, London, pp 843–850

Raemaekers M, Ramsey NF, Vink M, van den Heuvel MP, Kahn RS (2006) Brain activation during antisaccades in unaffected relatives of schizophrenic patients. Biol Psychiatry 59(6):530–535

Rajarethinam R, Upadhyaya A, Tsou P, Upadhyaya M, Keshavan MS (2007) Caudate volume in offspring of patients with schizophrenia. Br J Psychiatry 191:258–259

Schechter I, Butler PD, Silipo G, Zemon V, Javitt DC (2003) Magnocellular and parvocellular contributions to backward masking dysfunction in schizophrenia. Schizopr Res 64(2–3):91–101

Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S et al (2002) Neuregulin 1 and susceptibility to schizophrenia. Am J Hum Genet 71:877–892

Stefansson H, Sarginson J, Kong A, Yates P, Steinthorsdottir V, Gudfinnsson E et al (2003) Association of neuregulin 1 with schizophrenia confirmed in a Scottish population. Am J Hum Genet 72(1):83–87

Steinthorsdottir V, Stefansson H, Ghosh S, Birgisdottir B, Bjornsdottir S, Fasquel AC et al (2004) Multiple novel transcription initiation sites for NRG1. Gene 342(1):97–105

Thomson PA, Christoforou A, Morris SW, Adie E, Pickard BS, Porteous DJ et al (2007) Association of Neuregulin 1 with schizophrenia and bipolar disorder in a second cohort from the Scottish population. Mol Psychiatry 12(1):94–104

Tosato S, Dazzan P, Collier D (2005) Association between the neuregulin 1 gene and schizophrenia: a systematic review. Schizophr Bull 31:613–617

Viding E, Williamson DE, Hariri AR (2006) Developmental imaging genetics: challenges and promises for translational research. Dev Psychopathol 18(3):877–892 Review

Wechsler D (1999) Wechsler abbreviated scale of intelligence. Psychological Corporation, San Antonio, TX

Weinberger DR (1987) Implications of normal brain development for the pathogenesis of schizophrenia. Arch Gen Psychiatry 44(7):660–669

Woo RS, Li XM, Tao Y, Carpenter-Hyland E, Huang YZ, Weber J et al (2007) Neuregulin-1 enhances depolarization-induced GABA release. Neuron 54(4):599–610