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ORIGINAL PAPER

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Neuregulin 1 ICE-single nucleotide polymorphism in first episode schizophrenia correlates with cerebral activation in fronto-temporal areas

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Abstract The Neuregulin (NRG1) gene has been associated with schizophrenia, but its functional implications are largely unknown. Our aim was to assess differential brain activation between patients carrying an at-risk allele on the Neuregulin 1 gene and patients without this genetic risk. Neural signal changes between 14 first episode schizophrenia patients with the at risk allele (SNP8NRG221533) from the Icelandic core haplotype and 14 without were measured with fMRI during a working memory task. Patients without the at risk allele showed greater activations ($P < 0.05$; corrected) in the left hippocampus, precuneus and cerebellum, as well as the right anterior cingulate. Brain regions previously associated with the pathology of Schizophrenia are differentially affected in those with a genetic at risk status in the NRG1 gene. Heterogeneity of structural and functional measures within patients characterized by clinical phenotypes may be in part due to this genetic variation.

Key words neuregulin · schizophrenia · functional endophenotype · FMRI

Introduction

Schizophrenia is a prevalent neuropsychiatric disorder with a prominent genetic basis. Multiple genes interact during brain development and maturation, constituting the origin of an individual's risk for the disease. Stefansson and colleagues [25], integrating linkage, association and animal model-

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ling, reported evidence that Neuregulin 1 (NRG1) is one such susceptibility genes for schizophrenia. This has been confirmed since in several independent studies [3, 15, 24, 26, 27], although there have been negative reports as well [30]. The associated haplotypes are varying between investigated populations yet, the haplotype extracted from the original investigation (HAP-ICE) has been confirmed to be associated with schizophrenia in a recent systematic review including data from 4,500 subjects from several populations reporting positive associations to this gene region [33]. The association with schizophrenia of a SNP (SNP8NRG221533, risk allele C) within the HAP-ICE has further been replicated in a number of investigations (for review see Harrison and Law [11]) and a meta-analysis [14]. A pathogenic mutation in NRG1 has not yet been found however.

NRG1 is a large gene, giving rise to at least 15 peptides which are involved in diverse aspects of developmental biology, both in the brain and peripherally. Most importantly, it is implicated in the differentiation and myelination of Schwann cells and oligodendrocytes, the migration of CNS neuronal precursors along radial glia, synaptogenesis/-plasticity, and regulation of neurotransmitter receptors (NMDA, GABA, nicotinic acetylcholine receptors, for review see Falls [6]). The NRG1 family thus seems to play a major role in neurodevelopment, both during fetal gestation as well as postnatal reorganization and myelination processes, which continue until early adulthood. There is evidence that NRG1 signalling is altered in schizophrenia, because NRG1 gene expression profiles differ between patients and control subjects [32, 35].

Theoretical considerations [7] and a number of recent empirical studies [1, 2, 4, 16] suggest that cognitive endophenotypes, as assessed with behavioural or physiological methods such as functional magnetic resonance imaging (fMRI), may be highly sensitive to the functional effects of polymorphisms associated with schizophrenia, because these measures are closer to the gene effects than are diagnoses. To our knowledge, such functional effects of the SNP-ICE have not been described so far.

One of the main tasks used to study working memory functions and their neuronal correlates is the *n*-back version of the continuous performance task (CPT). It has been found that reaction time, hit rate, omission and commission errors are significantly impaired in patients with schizophrenia and their relatives when compared to healthy subjects (e.g., [36, 37]). Brain activation during this task typically comprises of bilateral frontal, prefrontal and parietal regions [38] in patients as well as in healthy subjects [39]. Studies comparing healthy individuals and schizophrenia patients show mixed results with regard to prefrontal and hippocampal activation patterns with hypo- as well as hyperactivations [40–42, 48]. Patients or their relatives when compared to healthy individuals. These activation patterns can be load- and performance dependent [43, 44]. In order to explain these divergent findings, it has been suggested that activation patterns follow an inverted u-shaped curve with decreased activation during very low and high task demands [45]. Nevertheless, studies with hypo- or hyperactivations in prefrontal, parietal or hippocampal areas are valid demonstrations of ineffective processes or compensatory mechanisms in order to perform working memory tasks [20]. Since these activation patterns are also found in relatives of patients, a genetic influence seems plausible. This is in line with findings on cognitive performance measures [46] and personality profiles [47] which are also under genetic influence.

Win the current study, we explored the influence of SNP8NRG221533 (risk allele C) on neural activation patterns. Patients with first episode schizophrenia were divided into groups with and without the risk allele. All subjects performed a working memory task (letter *n*-back task) while correlates of neural activation were measured with fMRI. Based on previous studies on the neural correlates of working memory in patients with schizophrenia versus healthy subjects [20], we predicted decreased activation in the risk versus non-risk groups in core regions previously associated with schizophrenia in post-mortem, structural and functional imaging studies that are also relevant for memory functions such as the prefrontal, anterior cingulate, lateral and medial temporal cortex.

Methods

Subjects

Thirty-eight first episode patients with DSM IV Schizophrenia [19] were recruited from the Departments of Psychiatry of the Universities Düsseldorf, Bonn, Cologne, Essen, Jena, Tübingen, and the Central Institute of Mental Health Mannheim, as part of the German Research Network of schizophrenia. All patients were genotyped and assessed with fMRI as described below. From this group 14 patients were carrying the at risk-SNP (SNP8NRG221533; risk allele (C)) within the HAP-ICE of [25]. These at risk patients were compared with 14 patients matched for age, sex and parental education that were not carrying the risk allele. All subjects were right handed [17] and native German speaking. Exclusion criteria for all subjects were past or present medical illness. Patients with psychiatric life time diagnosis other than schizophrenia were not included. Urine drug screening (for opioids, methadone, amphetamines, or cannabinoids) before fMRI measurements were negative in all participants. There were no significant differences between the patient subgroups on sociodemographic and clinical variables (Table 1). All participants were Caucasian.

Patients were diagnosed for schizophrenia by trained, experienced psychiatrists using a standardized clinical interview according to DSM-IV criteria (SCID; German Version, [34]; DSM IV [19]). After scanning, patients have been followed up over 2 years and hence a change of diagnosis could be excluded. Psy-

Table 1 Sociodemographic and psychopathological characteristics

Variable	Patient groups with and without risk allele (n = 28)		
	With risk n = 14	Without risk n = 14	Total n = 28
Age	32.4 ± 9.5	32.5 ± 10.8	32.4 ± 10
Sex (f:m)	7:7	7:7	14:14
Mean parental education (years)	11.4 ± 2.5	11.7 ± 2.8	11.6 ± 2.6
PANSS positive	10.2 ± 4.6	9.6 ± 4.3	9.9 ± 4.3
PANSS negative	13.3 ± 6.6	14.5 ± 6.2	13.9 ± 6.3
PANSS general	23.3 ± 12.4	24.1 ± 8.4	26.1 ± 10.5
CGI	2.8 ± 1.3	3.4 ± 1.2	3.1 ± 1.3
GAS	68.8 ± 10.5	63.1 ± 16	65.7 ± 13.8
HAMD	5.5 ± 5.7	4 ± 6.7	4.7 ± 6.2
Medication [number of patients and mean dose (mg) ± SD per day]	Risperidone (n = 7): 4.0 ± 1.4 Haloperidol (n = 7): 2.6 ± 1.0	Risperidone (n = 8): 2.3 ± 2.1 Haloperidol (n = 6): 3.0 ± 1.7	Risperidone (n = 15): 3.0 ± 2.0 Haloperidol (n = 13): 2.7 ± 1.2

There was no statistically significant difference in any of the variables within the two patient groups, i.e., between the risk versus non-risk carriers. Information on medication was unavailable in two patients

chopathological rating with the PANSS [13], Hamilton depression scale (HAMD [10]), global assessment of functioning (GAF [19]), clinical global impression (CGI [8]) revealed no significant differences between the patient groups (Table 1). All patients were on stable doses of neuroleptics, either haloperidol or risperidone (see Table 1). Patients represent a subsample of a larger group whose fMRI results have been reported elsewhere [20]. After a complete description of the study, written informed consent was obtained. The local ethics committees approved the protocol, which is in accordance to the declaration of Helsinki.

Genotyping

The DNA was isolated from whole blood or permanent cell lines derived from Epstein–Barr virus-transformed lymphocytes with a Qiagen Blood- and Cell-Culture kit; 12.5 ng of DNA was used for TaqMan genotyping assays. Genotyping of SNP8NRG221533 was performed using a Taqman Assay by Design (Applied Biosystems, Darmstadt, Germany) as described previously [21].

The Icelandic core SNP8NRG221533 (risk allele (C)) [25, 33] was selected to define the genetic at-risk states. The frequency of the constituent SNP8_221533 was 36.8% in our fMRI sample (14 out of 38 schizophrenic patients), closely matching its frequency in the Icelandic patient sample (36.4%).

Magnetic resonance imaging

Stimuli

The task during fMRI data acquisition consisted of an n-back paradigm with a randomized sequence of 0-back and 2-back conditions arranged in a block design. Subjects viewed single letters (A–Z, red on black background) in random order. Each letter appeared for 500 ms to which subjects had to react within 900 ms by pressing the response button (LUMItouch) for the target letter with their right index finger. The target probability for both conditions was 0.37 with a ratio of 48 non-targets to 28 targets. Baseline phases required subjects to only fixate the sequence of letters (without any response required). During '0-back', subjects also saw a sequence of letters and had to respond to a target letter (X). '2-back' required subjects to press the button when a letter occurred when the last but one letter had been the same. Subjects were instructed in detail with task examples on a computer before the experiment started and again during the experiment. The paradigm was applied in two runs with 99 whole brain acquisitions in each run, in which baseline and activation blocks alternated. Each run included four 0-back and four 2-back-trials and eight baselines of fixation, and had a duration of 8.25 min. Each block lasted for 30 s and comprised of six whole brain acquisitions.

Data acquisition

Cerebral activation was measured using fMRI, based on echo planar imaging (EPI) using BOLD contrast. FMRI brain images were acquired using Siemens and Phillips 1.5T MR scanners. The acquisition comprised of a 3D-dataset ($256 \times 256 \times 128$ sagittal, FOV 230 mm, TE = 4.4 ms, TR = 11.4 ms, $\alpha = 15°$) and transaxial functional images (EPI, 64×64, 32 slices, 3 mm thickness, FOV 200×200 mm^2, voxel size: $3.125 \times 3.125 \times 3$ mm^3, TE = 66 ms, TR = 5 s, $\alpha = 90°$), covering the whole brain and positioned parallel to the intercomissural line (AC-PC).

Quality control

Since the anticipated BOLD signal is small (<5% at 1.5 T) depending on the stimulus, quality control (QC) is important [22, 31]. In multi-center fMRI studies, QC is critical due to additional variance sources. Hence, QC of the hardware dependent noise contribution was carried out to investigate the influence of different scanners contributing to the data variance. This had to be verified individually for each measurement and site. The fMRI hardware was tested after each in vivo measurement by running the same EPI sequence (1 run with 99 whole brain acquisitions) on the standard SIEMENS phantom. The exact method has been described in detail previously [28].

However, the phantom measurements control for scanner hard- and software quality are not suitable to estimate in vivo data quality and exclude functional data sets since the latter is dominated by other effects such as movement and physiological noise. In order to eliminate corrupted measurements and to reduce the noise of fMRI results, the quality of each in vivo data set was estimated using the mean deviation of the time-series from its mean image, expressed in percentage signal change (PSC). Exact details of the calculation procedure can again be found in [28]. None of the data-sets used here exceeded the previously chosen threshold for the PSC-value of <3.4 [20] .

Data analysis

Behavioral data

Accuracy (percent correct) was the main dependent measure. Number of responses and reaction time were also assessed. Group differences were analyzed with separate two-way repeated measure ANOVAs with between-subject factor group (patients with and without risk) and within-subject factor task (2-back, 0-back) for each variable (results see Table 2).

fMRI

Data analysis was performed using SPM2 (http://www.fil.ion.ucl.ac.uk/spm). The first three images were discarded. After realignment, co-registration, stereotaxic normalization and smoothing (10 mm isotropic Gaussian filter), statistical parametric maps were calculated independently for each subject using a delayed boxcar convolved with a hemodynamic response function. Contrast images for each subject were created contrasting the 2-back with the 0-back condition focusing on working memory. The group analysis was based on a random effects model, applying one sample *t* tests per group per contrast (threshold at $P = 0.05$, corrected for multiple comparisons, results see Tables 3, 4). Between-group comparisons were performed on the contrast 2-back versus 0-back with a two-sample *t* test. In order to correct for multiple comparisons within a search volume we applied a cluster extent threshold determined by Monte Carlo simulations [23]. In the Monte-Carlo procedure "sufficient" data sets under the null hypothesis are simulated. Then an (arbitrary) uncorrected threshold is applied to each simulated 3D image and the number of voxels for a cluster to be considered active is increased stepwise. The desired extent threshold is then established by determining the minimal cluster size (or extent threshold) for which maximally 100 × alpha percent of the data sets find a positive result. Here alpha is the pre-specified type I error rate, which is conventionally set to 0.05. For a threshold at the voxel level at 0.05 and spatial properties as present in this study, 1,000 simulations resulted in an extent threshold of 59 resampled voxels. This procedure prevented a false positive rate above 5% due to multiple testing (results see Table 5).

In order to visualize the scanner variability we plotted the mean activation of the between group analysis of a sphere with 10 mm diameter around the global maximum of the risk allele sample. Subsequent non-sphericity correction did not change the results (see Fig. 2).

Table 2 Behavioral performance

	Mean ± SD	
	0-Back	2-Back
Accuracy (% correct)		
Patients with risk allele (C)	95.1 ± 12.8	87.1 ± 17.1
Patients without risk allele (C)	96.6 ± 5.4	88.5 ± 9
Reaction time (ms)		
Patients with risk allele (C)	617.6 ± 240.7	691.8 ± 181.1
Patients without risk allele (C)	527.3 ± 115.3	566.5 ± 124

Means and standard deviations of performance of patients. There was no statistical difference between groups

Table 3 Brain activation (one sample *t* test) in patients without genetic risk (FDR corrected, $P = 0.05$, extent threshold $k > 10$ voxels)

Anatomical region[a]	Hemisphere	MNI (*x*,*y*,*z*)	*k*	*Z*
Middle frontal gyrus	Right	36 54 2	75	4.68
Frontal inferior operculum	Left	−42 4 24	797	4.62
Insula	Left	−30 22 −8	228	4.39
Superior parietal lobe	Right	22 −70 56	342	4.36
Supramarginal gyrus	Right	42 −46 34	167	4.27
Frontal inferior operculum	Right	32 26 −6	119	4.20
Inferior frontal gyrus (pars triangularis)	Right	46 14 28	160	4.05
Fusiformgyrus	Left	−42 −66 −20	229	4.01
Superior parietal lobe	Left	−18 −64 48	397	3.92
Middle frontal gyrus	Right	−36 10 52	268	3.91
Medial superior frontal gyrus	Right	0 24 42	94	3.82
Middle frontal gyrus	Left	−26 50 −2	18	3.69
Inferior frontal gyrus (pars triangularis)	Right	46 32 26	63	3.69
Middle occipital gyrus	Right	32 −94 −6	15	3.67
Inferior parietal lobe	Left	−52 −48 44	123	3.64
Inferior parietal lobe	Left	−40 −40 38	18	3.38

[a]Anatomical region according to the Anatomical Automatic Labeling (AAL) for SPM2: http://www.cyceron.fr/freeware/

Table 4 Brain activation (one sample *t* test) in patients with genetic risk (FDR corrected, $P = 0.05$, extent threshold $k > 10$ voxels)

Anatomical region[a]	Hemisphere	MNI (*x*,*y*,*z*)	*k*	*Z*
Inferior parietal lobe	Right	38 −52 44	2,548	5.10
Precentral gyrus	Right	46 8 50	7,054	5.02
Inferior parietal lobe	Left	−42 −42 48	2,119	4.98
Middle frontal gyrus (pars orbicularis)	Right	36 56 −4	244	4.06
Middle frontal gyrus	Right	36 26 48	115	3.82
Superior frontal gyrus	Left	−34 60 12	186	3.40
Inferior temporal gyrus	Right	56 −56 −16	29	3.39
Lingual gyrus	Left	−38 −78 −18	40	3.36
Caudate	Right	18 2 20	29	3.30
Supplementary motor area	Left	−10 −14 68	14	3.20
Fusiform gyrus	Right	42 −80 −14	28	3.11
Insula	Left	−42 18 −8	25	3.01

[a]Anatomical region according to the Anatomical Automatic Labeling (AAL) for SPM2: http://www.cyceron.fr/freeware/

Table 5 Difference of cerebral signal changes measured with fMRI in 14 patients without risk allele (C) versus 14 patients with this genetic risk contrasting 2-back minus 0-back conditions (uncorrected height threshold, $P = 0.05$; extent threshold, $k > 59$ voxels)

Anatomical region[a]	Hemisphere	MNI (*x*,*y*,*z*)	*k*	*Z*
Precuneus	Left	−16 −70 40	163	2.91
Anterior cingulate	Right	12 32 −2	91	2.80
Lingual gyrus	Right	4 −72 −2	102	2.29
Hippocampus	Left	−40 −6 −20	119	2.24
Cerebellum	Left	−16 −24 −26	61	1.89

The opposite contrast showed no activated voxels

[a]Anatomical region according to the Anatomical Automatic Labeling (AAL) for SPM2: http://www.cyceron.fr/freeware/

Results

Behavioral data

The two-way ANOVA (group, task) for accuracy and reaction time revealed no significant main effect for

group and no significant group × task interaction (for details see Table 2).

FMRI data

Neural correlates of working memory load was operationalised as contrast between the 2-back and the 0-back condition. The one sample *t* test in each group (with and without genetic risk; FDR corrected, $P < 0.05$) showed the most pronounced activations in frontoparietal areas known to be involved in such an *n*-back task (see Tables 3, 4).

We compared carriers and non-carriers of the at-risk allele with a two-sample *t* test. Patients without the at-risk allele showed significantly stronger activations ($P < 0.05$; cluster extent of 59 voxels) in the left precuneus, the right anterior cingulate gyrus, the right lingual gyrus, the left hippocampus and the left cerebellum (see Table 4, Fig. 1).

In two post hoc tests, we used the medication dose (mg/day) and the reaction times as covariates of no interest in the analysis. The results did not change.

To rule out effects of 'study center' we plotted mean signal changes of the between group analysis for each patient of the two groups (with and without risk allele), using all regions as ROI. There was no effect of 'center' (Fig. 2).

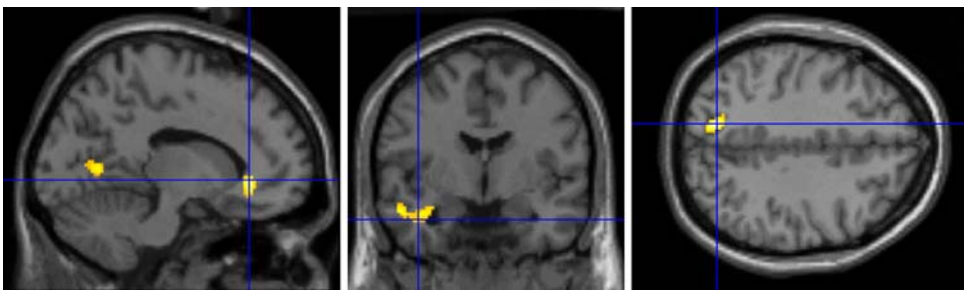

Fig. 1 Significant group differences in fMRI activation (two-sample *t* test) for the contrast 2-back versus 0-back between patients without risk versus patients with genetic risk (uncorrected height threshold, $P = 0.05$; extent threshold, $k = 59$ voxels) showing increased activations in patients without risk allele ($n = 14$) compared to patients with this risk ($n = 14$) in the right anterior cingulate gyrus, the right lingual gyrus, the left hippocampus and the left precuneus (projections onto a standard brain)

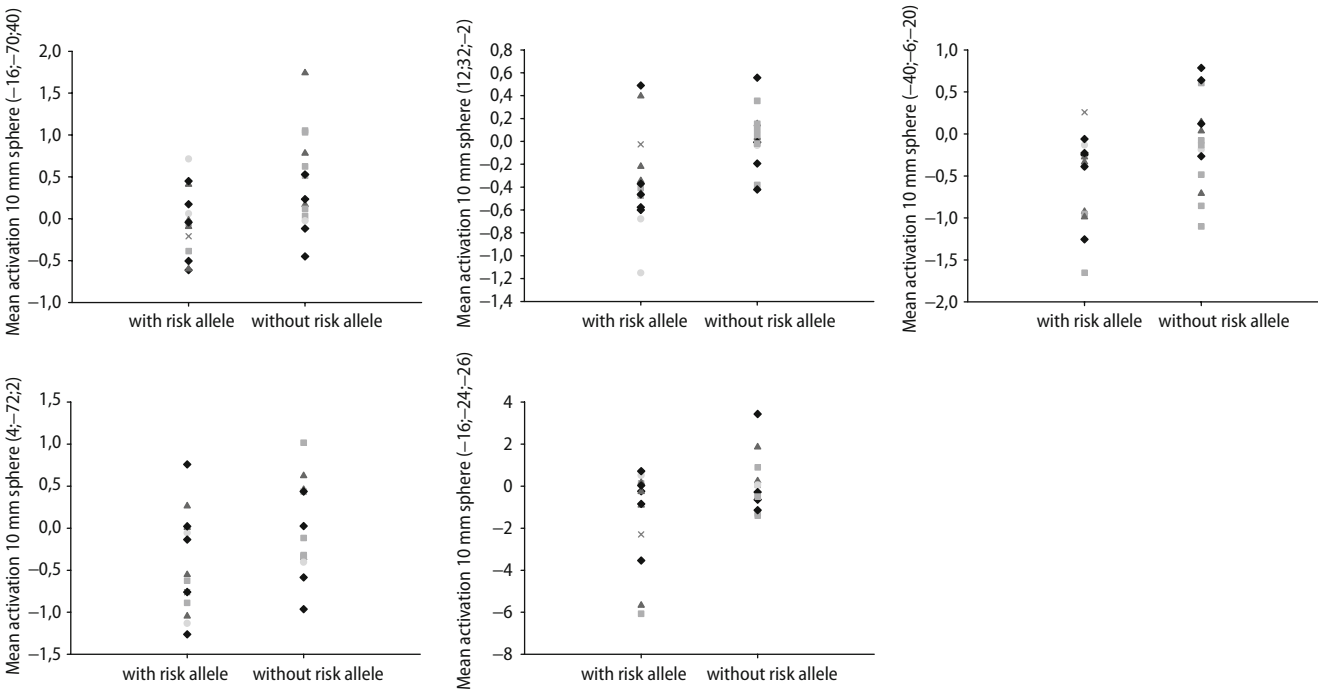

Fig. 2 Mean activations in 10 mm spheres around the a priori hypothesized local differences in maximum signal changes between risk and non-risk allele carriers. Coordinates (*x*, *y*, *z*) taken from the between group analysis are shown at the *y*-axis of each graph for every patient for each study center (14 subjects carrying the risk allele on the *left* side and 14 patients without this risk on the *right* side of the graphs). *Different symbols* are used for each center. Notice the variation according to the genetic risk independent of the center. The results point to non-sphericity (cf. variance in the group without risk allele). The appropriate correction for non-sphericity did not change the results

Discussion

In our study we compared neural activation measured with fMRI during a working memory task in first episode patients with schizophrenia carrying the SNP8NRG221533 NRG1 at-risk SNP (risk allele C) to non-carriers. The risk allele carriers revealed decreased activation in cortical key areas for schizophrenia such as the anterior cingulate, hippocampus and precuneus. These regions have repeatedly been associated with the pathophysiology of schizophrenia, as demonstrated by numerous post-mortem, structural and functional imaging studies. The NRG1 gene seems thus to play an important role in the structure, function and/or connectivity among these cortical areas. We could demonstrate that heterogeneity of structural and functional measures within schizophrenia patients characterized solely by clinical phenotypes may be in part due to genetic variation in the NRG1 gene.

Only few studies thus far have tried to link genetic polymorphisms of schizophrenia susceptibility genes to brain function or structure in control subjects [1, 4, 5] or patients with schizophrenia [12, 16, 18, 29]. These data, in conjunction with our own, point to modulatory and potentially additive effects of different polymorphisms in the pathophysiology of schizophrenia. In particular, Hall et al. [9] have demonstrated differences in brain activation in high risk subjects for schizophrenia, depending on their NRG1 genotype. They however used a different cognitive task and genotyped for another SNP. Their task consisted of a sentence completion paradigm, where a given sentence stem had to be completed with either a fitting or non-fitting word. As a tagging marker they used SNP8NRG243177. Subjects with the risk (T/T) genotype showed significantly decreased activation of medial prefrontal cortex (Brodmann area 9) and right temporo-occipital junction (Brodmann areas 39 and 19) relative to those without the risk allele in the contrast between activation during sentence completion and activation during rest. Although subjects (patients in our study vs. high risk subjects in their study), cognitive task and tagging marker were different, it is interesting that both studies found activation differences in the medial frontal cortex. However due to the differences in methodology, it is difficult to directly compare the studies further. Their and our data point in the same direction as cerebral signal changes are being modulated by NRG1 genotype.

Since our patients were recruited during their first episode, we could rule out potential long term degenerative changes due to the course of the disorder. The within group analysis showed a typical activation pattern during verbal working memory tasks and replicates earlier findings in a larger group of first episode patients [20]. An automated QC procedure was applied to detect outliers and therefore to ensure noise homogeneity between groups in the second level analysis [28]. The approach eliminates high data variability due to artefacts, possibly induced by the fMRI hardware or the subject. None of the data sets in our sample had to be excluded.

Multi-center fMRI studies are still a novelty but recent results have demonstrated their feasibility [20]. In our study, results were independent of the study center, as shown in our ROI analysis. The results may be somewhat constrained by the sample size in our analysis, however they were in line with our hypothesis. The activation differences were localized in key regions of schizophrenia, thus confirming our initial hypothesis and do therefore qualify for further investigation.

We have to consider treatment with neuroleptic medication and small sample size as limitations of our study. Patients received only Risperidone or Haloperidol and the number of patients treated with each drug as well as doses were comparable in each group. We compared two groups of patients, so the influence of medication should be similar in both samples. To rule out confounding factors, we used the medication dose (mg/day) and the reaction times as covariates of no interest in two different post hoc tests into the analysis. The results did not change.

Conclusion

We have demonstrated differential activation in cerebral key regions implicated in schizophrenia depending on the genetic at risk status. Within phenotypically characterized schizophrenia the NRG1 gene seems to exert modulatory influence on brain function. These findings encourage further search for functional endophenotypes as a possible future tool for early detection of individuals at risk for schizophrenia. The present results should be independently replicated in a larger sample to establish the role of fMRI activation studies in the genetic background of schizophrenia.

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