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Polygenic risk for schizophrenia affects working memory and its neural correlates in healthy subjects

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ABSTRACT

Schizophrenia is a disorder with a high heritability. Patients as well as their first degree relatives display lower levels of performance in a number of cognitive domains compared to subjects without genetic risk. Several studies could link these aberrations to single genetic variants, however, only recently, polygenic risk scores as proxies for genetic risk have been associated with cognitive domains and their neural correlates.
In the present study, a sample of healthy subjects ($n = 137$) performed a letter version of the n-back task while scanned with 3-T fMRI. All subjects were genotyped with the PsychChip and polygenic risk scores were calculated based on the PGC2 schizophrenia GWAS results.
Polygenic risk for schizophrenia was associated with a lower degree of brain activation in prefrontal areas during the 3-back compared to the 0-back baseline condition. Furthermore, polygenic risk was associated with lower levels of brain activation in the right inferior frontal gyrus during the 3-back compared to a 2-back condition.
Polygenic risk leads to a shift in the underlying activation pattern to the left side, thus resembling results reported in patients with schizophrenia. The data may point to polygenic risk for schizophrenia being associated with brain function in a cognitive task known to be impaired in patients and their relatives.

1. Introduction

Schizophrenia is a severe disorder with a high heritability (Sullivan et al., 2003). Deficits in a number of neuropsychological domains such as episodic memory, verbal fluency and working memory have been described in schizophrenia patients (Heinrichs and Zakzanis, 1998; McCleery et al., 2014; Zhang et al., 2015) as well as their first degree relatives (Jabben et al., 2010; Kristian Hill et al., 2015). It has been shown that performance in different cognitive domains as well as neural correlates of these functions are associated with several molecular genetic variants that are also associated with the disorder. It could be shown that e.g. verbal fluency (Krug et al., 2010b), episodic memory (Krug et al., 2010a, 2013) as well as working memory (Krug et al., 2008) are affected by such variants. However, these studies only explained small portions of variance. Also the genetic variants increase risk by a very small amount.

The neural correlates of working memory typically encompass the bilateral prefrontal cortex as well as bilateral temporal and parietal cortices (Markov et al., 2010). In schizophrenia, it has been shown that these networks are activated to a lesser degree compared to healthy subjects (e.g. (Koike et al., 2013; Sugranyes et al., 2012)). However, reverse results have also been reported (Brandt et al., 2014) which lead to the hypothesis of an inverted u-shaped curve of prefrontal activation as a function of task difficulty (Manoach, 2003). It has been postulated that this curve is shifted to the left in patients, leading to a decrease in activation earlier (during easier conditions) than in controls. In a previous study, it was shown that neural activation of the left frontal cortex (Brodman area 10) was linearly correlated with the number of risk alleles in *NRG1*, confirming part of this hypothesis (Krug et al., 2008).

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However, only a 2-back version of the n-back task was used in this latter study, which proved too easy for healthy subjects to display signs of decreasing activation due to difficulty.

As single variants typically only explain small portions of variance in neural activation, recent studies employed polygenic risk scores to investigate genetic effects on neural correlates of a variety of cognitive domains such as executive functioning (Whalley et al., 2015), working memory (Kauppi et al., 2015; Walton et al., 2013, 2014), as well as brain morphology (Terwisscha van Scheltinga et al., 2013) and symptom dimensions (Derks et al., 2012). These studies presented first evidence that these domains are associated with polygenic risk. However, within these studies, only tasks leading to ceiling effects in healthy subjects were investigated (e.g. 2-back and 0-back conditions in the n-back task, (Kauppi et al., 2015)). To test whether polygenic risk affects the hypothesized u-shaped curve of prefrontal activation, tasks that span the whole range of difficulty levels have to be tested.

The aim of the present study was to investigate the association of a polygenic risk score for schizophrenia with the neural correlates of working memory using a version of the n-back task with a 2-back and a 3-back condition in healthy subjects. The 3-back condition was added to raise difficulty as a 2-back version alone might have been too easy for healthy subjects. Healthy subjects were chosen to minimize effects of the disorder itself as well as medication status. It was hypothesized that activation of the network underlying this paradigm would be negatively correlated with the risk score. This should be especially true for the 3-back condition (compared to baseline or 2-back condition) as it should be more demanding than the 2-back condition.

2. Material and methods

2.1. Subjects

Subjects were recruited via postings in Marburg, Germany. No subject reported any psychiatric disorder according to ICD-10 past or present as tested with the SCID-I interview (performed by trained psychologists). In total, 137 subjects (77 men), mean age 34.52 (SD = 10.46), with a mean education of 13.3 (SD = 3.75) years participated in this study. All subjects were of western European descent and native German speakers. When asked, subjects denied any family history of schizophrenia. The protocol was approved by the local ethics committees according to the declaration of Helsinki.

2.2. DNA extraction, genotyping and quality control

Genomic DNA was extracted from ethylenediaminetetraacetic acid (EDTA) anticoagulated venous blood according to standard procedures. All individuals were genotyped on the Infinium PsychArray BeadChip (PsychChip, Illumina, San Diego, CA, USA). Genotyping was performed at the Department of Genomics, Life & Brain Center, University of Bonn, Germany. Genotypes were called using the GenomeStudio software and imputed to the 1000 Genomes reference data phase 1, version 3, using a custom imputation pipeline which is available at: https://github.com/CNSGenomics/impute-pipe. Individuals were excluded if they had a genotyping call rate lower than 95%, conflicting sex information, represented population outliers in the first two ancestry principal components (HapMap3 CEU population mean ± 7 standard deviations) or exhibited relatedness >0.05 to other individuals in the sample.

2.3. Assessment of polygenic risk score

Schizophrenia polygenic risk scores were calculated using the PGC2 schizophrenia GWAS results (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), obtained from http://www.med.unc.edu/pgc/downloads (file scz2.prs.txt.gz). In order to remove SNPs that are in high linkage disequilibrium (LD) with one another, clumping was performed on the genotype data with respect to the schizophrenia association *p*-values, using an LD r2 threshold of 0.2 and physical distance threshold of 50 kb. A p-value threshold of 0.05 was applied after clumping for selection of SNPs for the calculation of polygenic risk scores. After clumping, the 637,186 remaining SNPs were used to calculate polygenic risk scores. PLINK 1.9 was used both for clumping and score calculation (https://www.cog-genomics.org/plink2, GigaScience 2015, (Chang et al., 2015).

2.4. Neuropsychological testing

All Subjects were tested with the letter-number-span (verbal working memory, (Gold et al., 1997) the spatial-span (spatial working memory, (Wechsler, 1997)) and the MWT-B (verbal intelligence, (Lehrl et al., 1995)). Scores were analysed with partial correlation analyses implemented in SPSS 20. Correlations with polygenetic risk scores were controlled for age and sex.

2.5. fMRI data acquisition

FMRI data was acquired with a 3 Tesla Tim Trio MR scanner (Siemens Medical Systems) at the Department of Psychiatry and Psychotherapy, Philipps-University Marburg. A T2*-weighted echo-planar imaging (EPI) sequence sensitive to BOLD contrast (64 * 64 matrix, 224 mm * 224 mm FoV, 40 slices, 3.5 mm slice thickness, TR = 2.5 s, TE = 30 ms, flip angle = 90°) was used for the functional data. The slices covered the whole brain and were positioned transaxially parallel to the anterior–posterior commissural line (AC–PC). To eliminate the influence of the T1 stabilization effects we excluded the initial three of 160 gathered functional images from further analysis.

2.6. N-back task and fMRI-procedure

Presentation software package was used for presenting the stimuli in the MRI-scanner. The n-back task consisted of four different conditions: 0-back (0b), 1-back (1b), 2-back (2b) and 3-back (3b). At the beginning of each trial the particular condition was presented in the middle of the screen. The participants had to press either the left or the right button every time they saw a letter on the monitor. The left button was operated with the right index finger and the right button was to be pressed with the right middle finger. The letters where capitalized and presented as single white letters on a black background for 1 s each with a black screen between letters (500 ms). Each condition was presented twice for 30 s in a pseudo-randomized order. Every block featured 12 stimuli which included 4 target stimuli. This was true for all conditions except the 3-back condition which contained 3 target stimuli only. For the 0-back condition participants had to press the right button every time letter X was presented. If any other letter appeared, they had to press the left button. In the 1-back condition subjects also had to press the left button for each letter, except if the presented character was identical to the last presented one. The 2- and 3-back condition were identical, except that participants had to press the right button whenever they saw the identical letter presented 2 or 3 characters ago, respectively. Participants were instructed in detail before the experiment, with an additional short briefing when laying in the MRI-scanner before the experiment started.

2.7. fMRI data analyses

The fMRI data was analyzed using the using SPM8 standard routines and templates (Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm) running on MATLAB 7.7.0.471 (R2008b) (The MathWorks, Inc.). Functional images were realigned and normalised to standard MNI (Montreal Neurological Institute) space (resulting voxel size: 2 mm × 2 mm × 2 mm). To increase the signal-to-noise ratio and to compensate for inter-subject anatomical variation, functional scans were spatially smoothed with an 8 mm full-width-at-half-

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