Retraction and Correction

RETRACTION

SYSTEMS BIOLOGY


The authors wish to note the following: “In this paper we report an association of the ‘synapse organization and biogenesis’ gene set with a neuroimaging phenotype, using gene set enrichment methodology. The methods and results of the paper, as described, have been conducted after consultation with experts in the field and support this conclusion. However, a potential confound relating to statistical inference has been brought to our attention that arises from the fact that several clustered genes, all of which are included in this gene set, have been tagged by the same SNP. This problem, which concerns only a small fraction of our tested gene sets (unfortunately including our top finding), belongs to a known category of potential pitfalls in gene set association analyses, and we are sorry that this problem was not detected earlier. Our reanalyses suggest that if adjustments for this confound are applied, the results for our top finding no longer reach experiment-wide significance. Therefore, we feel that the presented findings are not currently sufficiently robust to provide definitive support for the conclusions of our paper, and that an extensive reanalysis of the data is required. The authors have therefore unanimously decided to retract this paper at this time.”

Luanna Dixson
Henrik Walter
Michael Schneider
Susanne Erk
Axel Schäfer
Leila Haddad
Oliver Grimm
Manuel Mattheisen
Markus M. Nöthen
Sven Cichon
Stephanie H. Witt
Marcella Rietschel
Sebastian Mohrke
Nina Seifertth
Andreas Heinz
Heike Tost
Andreas Meyer-Lindenberg

CORRECTION

CELL BIOLOGY


The authors note that the author name Constantinescu should instead appear as Constantinescu. The corrected author line appears below. The online version has been corrected.

Casmir Turnquist, Yihua Wang, David T. Severson, Shan Zhong, Bin Sun, Jingyi Ma, Stefan N. Constantinescu, Olaf Ansorge, Helen B. Stolp, Zoltán Molnár, Francis G. Szele, and Xin Lu

www.pnas.org/cgi/doi/10.1073/pnas.1415682111
Identification of gene ontologies linked to prefrontal–hippocampal functional coupling in the human brain

Luanna Dixson,a,b 1 Henrik Walter,b,c 1 Michael Schneider, a Susanne Erk, b Axel Schäfer, b Leila Haddad, b Oliver Grimm, b Manuel Mattheisen,d,e Markus M. Nöthen,d,e Sven Cichon,d Stephanie H. Witt,d Marcella Rietschel, a Sebastian Mohnke, e Nina Seifert,b Andreas Heinzb, Heike Tost,b,1 and Andreas Meyer-Lindenberg a,b 1

Departments of a Psychiatry and Psychotherapy and b Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, 68159 Mannheim, Germany; b Department of Psychiatry, Division of Mind and Brain Research, Charité Campus Mitte, 10117 Berlin, Germany; c Department of Genomics, Life and Brain Center, d Institute of Human Genetics, and e Institute for Genomic Mathematics, University of Bonn, 53127 Bonn, Germany

Edited by Robert Desimone, Massachusetts Institute of Technology, Cambridge, MA, and approved May 16, 2014 (received for review March 5, 2014)

Functional interactions between the dorsolateral prefrontal cortex and hippocampus during working memory have been studied extensively as an intermediate phenotype for schizophrenia. Coupling abnormalities have been found in patients, their unaffected siblings, and carriers of common genetic variants associated with schizophrenia, but the global genetic architecture of this imaging phenotype is unclear. To achieve genome-wide hypothesis-free identification of genes and pathways associated with prefrontal–hippocampal interactions, we combined gene set enrichment analysis with whole-genome genotyping and functional magnetic resonance imaging data from 269 healthy German volunteers. We found significant enrichment of the synapse organization and biogenesis gene set. This gene set included known schizophrenia risk genes, such as neural cell adhesion molecule (NRCAM) and calcium channel, voltage-dependent, beta 2 subunit (CACNB2), as well as genes with well-defined roles in neurodevelopmental and plasticity processes that are dysfunctional in schizophrenia and have mechanistic links to prefrontal–hippocampal functional interactions. Our results demonstrate a readily generalizable approach that can be used to identify the neurogenetic basis of systems-level phenotypes. Moreover, our findings identify gene sets in which genetic variation may contribute to disease risk through altered prefrontal–hippocampal functional interactions and suggest a link to both ongoing and developmental synaptic plasticity.

Imaging genetics is widely used to identify neural circuits linked to genetic risk for heritable neuropsychiatric disorders, such as schizophrenia, autism, or bipolar disorder (1). A well-established imaging genetics phenotype is functional connectivity between the right dorsolateral prefrontal cortex (DLPFC) and the left hippocampus (HC) during working memory (WM) performance (2–4). Specifically, impaired interaction of the HC and prefrontal cortex (PFC) has been proposed as a core abnormality during neurodevelopment in schizophrenia. The hippocampus provides input to the DLPFC through long-range glutamatergic connections, which have been linked to the glutamate hypothesis of the illness. Moreover, selective lesions of the hippocampus in primates and rodents have been shown to result in postpubescent changes in prefrontal regions that are consistent with neuropathological findings in schizophrenic patients (5, 6). Brain physiology during WM performance is highly heritable (7), and anomalies of prefrontal–hippocampal functional coupling during WM have been identified in schizophrenic patients (1, 2, 4, 8), their unaffected first-grade relatives (4), healthy carriers of genome-wide supported schizophrenia risk variants and subjects at risk (4, 9–12), and in genetic animal models of the disorder (13). These studies provide strong support for a role of this neural systems-level phenotype in schizophrenia pathophysiology and correspond well to current theories that conceptualize the illness as a “brain disconnection syndrome” rooted in disturbed synaptic plasticity processes (14, 15).

Previous studies have characterized abnormal prefrontal–hippocampal interactions in subjects with genetic risk factors for schizophrenia (4, 9, 10, 16). In particular, genome-wide association studies (GWAS) have become a standard approach for identifying common variants that may contribute to risk phenotypes in structural and functional neuroimaging data (10, 16, 17). However, although this approach has been effective in identifying genetic risk variants for imaging phenotypes, post hoc interpretation of results is challenging. Detected risk variants often fall within intronic sequences, where a lack of prior knowledge on functionality hinders a mechanistic explanation of how they impact brain function (18).

Increasing evidence suggests that common genetic risk variants for psychiatric disorders are not distributed randomly but rather lie among sets of genes with overlapping functions (19–22). Gene set enrichment analysis (GSEA) is a data analytical approach that leverages a priori knowledge to gain insight into the biological functions and pathways in the analysis of genetic data (23, 24). This approach relies on analysis of sets of genes grouped by common biological characteristics, such as a shared role in particular molecular functions or metabolic pathways. GSEA can then be used to test whether genes that are more strongly associated with functional connectivity | GSEA | endophenotype | genetic risk variants

Significance

This study combines neuroimaging and whole-genome genotyping techniques with a gene set enrichment analysis to unravel the genetic basis of a well-validated intermediate phenotype for schizophrenia, dorsolateral prefrontal cortex–hippocampal connectivity. We found significant enrichment of genes with roles in synaptic plasticity and neurodevelopment that are consistent with the neurobiological basis of prefrontal–hippocampal interactions in schizophrenia. We further provide additional independent evidence for the intermediate phenotype concept and present a readily generalizable approach for a biologically driven analysis of imaging and genetic data.


Conflict of interest statement: A.M.-L. has received consultant fees and travel expenses from Alexza Pharmaceuticals, AstraZeneca, Bristol-Myers Squibb, Defined Health, Decision Resources, Desitin Arzneimittel,Elsevier,F.Hoffmann-La Roche,Gerson Lehrman Group,Grupof Ferrer,Les Laboratoires Servier,Lilly Deutschland,Lundbeck Foundation,Outcome Sciences,OutcomeEurope,PriceSpective,and Roche Pharma and has received speaker’s fees from Abbott,AstraZeneca,BASF,Bristol-Myers Squibb,GlaxoSmithKline,Janssen-Cilag,Lundbeck,Pfizer Pharma, and Servier Deutschland.No other disclosures were reported.

This article is a PNAS Direct Submission.

1L.D., H.W., H.T., and A.M.-L. contributed equally to this work.

2To whom correspondence should be addressed. E-mail: a.meyer-lindenberg@zi-mannheim.de.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1404082111/-/DCSupplemental.
a phenotype of interest tend to significantly aggregate within specific biologically based “gene sets.” As an adjunct to established GWA studies and candidate gene approaches, GSEA has successfully identified gene sets with established risk genes for complex diseases such as lung cancer, Parkinson’s disease, and psychiatric disorders, yielding insight into plausible biological processes and molecular mechanisms warranting further investigation (24–26).

Although in principle the same strategy can be applied to other quantitative risk-associated phenotypes (27), no prior study has attempted to identify shared biological pathways linked to individual variation in DLPFC–HC functional coupling through a combination of GSEA, whole-genome genotype data, and neuroimaging. Here we used GSEA to test the association of ontology-based gene sets derived from common genetic variants with prefrontal–hippocampal interactions in 269 healthy volunteers who performed the n-back WM task during functional magnetic resonance imaging (fMRI), a well-established paradigm to challenge DLPFC–HC interactions. Given the reviewed evidence (14, 15), we hypothesized that we would identify gene sets linked to developmental plasticity and synaptic neurotransmission, including previously identified risk genes for schizophrenia.

Results
Consistent with previous studies (2, 9, 28, 29), healthy subjects showed robust activation increases in the 2-back condition relative to the 0-back condition in cortical areas linked to WM performance, including the right DLPFC (Fig. 1A). Additionally, our functional connectivity analysis with the right DLPFC seed revealed a relatively uniform negative connectivity with the left hippocampus (Fig. 1B).

GSEA of genome-wide contributions to the DLPFC–HC coupling phenotype revealed significant enrichment of the “synapse organization and biogenesis” category within the biological process ontology. Genes that contributed to the core enrichment signal of this gene set included genes previously implicated in schizophrenia pathophysiology, such as neuroligin 1 (NLGN1) (Table 1). This category was significant after correction for comparison of multiple gene sets using the false discovery rate (FDR q value = 0.04) and after conservative family-wise error correction (FWE P value = 0.04). The synapse organization and biogenesis category is also modest in size, with 23 genes, indicating that it maps to a lower, “more specialized” level of the gene ontology graph. Further details about this significant category, including HUGO Gene Nomenclature Committee gene symbols (30), full gene names, gene association P values, position of each gene on the ranked gene list, and gene enrichment scores, are shown in Table 1.

Discussion
GSEA analysis of the biological process ontology revealed a significant enrichment of genes encoding proteins integral to the formation, maintenance, and function of synapses in the brain. Genes within this category have been associated with schizophrenia risk in previous studies and impact several downstream processes and signaling pathways, including cellular adhesion and trans-synaptic signaling processes [protocadherin genes (PCDHs), NLGN1, neural cell adhesion molecule gene (NRCAM), agrin gene (AGRN)], organization and function of synaptic cytomatrix and scaffold complexes [AGRN, collagen type IV alpha 4 gene (COL4A4)], calcium signaling [voltage-dependent calcium channel beta 2 subunit gene (CACNB2), protein-cadherin beta 10 gene (PCDH10)], and ion channel and neurotransmitter function (CACNB2, nicotinic cholinergic receptor alpha 1 subunit gene (CHRNA1), acetylcholinesterase gene (ACHE)). Therefore, our findings both shed light on the underlying genetic architecture of the systems-level phenotype we studied and suggest ways in which abnormalities in the interaction between the PFC and HC may be relevant to genetic risk for schizophrenia.

The establishment and maturation of appropriate synaptic connections is crucial for neural circuit development and plasticity in the developing and mature brain. There is persuasive evidence linking dysfunction at the synapse level to schizophrenia pathophysiology (14, 31–34). Alterations to synaptic microcircuitry within the DLPFC of patients with schizophrenia include reduced excitatory inputs at layer 3 pyramidal neurons, increases in neuronal density, and altered expression of synaptic proteins (31, 35). In parallel, altered functional plasticity processes, such as long-term potentiation, which form the molecular basis of learning and memory, are disrupted in animal genetic and behavioral models of schizophrenia (14, 31). Our data add to a growing body of genetic, structural, and functional evidence implicating perturbed synaptic plasticity processes in altered prefrontal network dynamics observed in schizophrenic patients (3, 34). A study of cortical thickness in patients with schizophrenia found decreases in the structural connectivity of the left and right DLPFC to be correlated with poor WM performance (36). Additionally, real-time transcranial magnetic stimulation techniques, which allow researchers to indirectly study synaptic plasticity in vivo (37, 38), have shown that inducing synaptic plasticity in the right DLPFC leads to altered prefrontal–hippocampal functional interactions during WM (3). These findings are in keeping with the “two hit” hypothesis of schizophrenia, whereby early environmental and genetic factors confer vulnerability of neural circuitry to adverse events during adolescence, such as excessive synaptic pruning or impaired plasticity (14, 39). Diminished stabilization of these circuits is thought to contribute to enduring functional and structural alterations in the underlying neurocircuitry, leading to the altered experience-dependent plasticity and connectivity features that are associated with the cognitive and behavioral symptoms of schizophrenia. Finally, our data are highly consistent with a growing body of pathway analytic analyses that provide support for a role of synaptic gene groups in the pathophysiology of schizophrenia (22, 40–42).

Adhesion. Among the genes included in the synapse organization and biogenesis category are several cell adhesion genes, from the protocadherin, neuroligin, and neural adhesion gene families. Synapses in the central nervous system are formed through a series of reciprocal adhesion events between axons and corresponding dendrites that regulate contact initiation, synapse formation, maturation, and functional plasticity. Cell adhesion genes are gaining

Fig. 1. (A) WM-related BOLD activation (2-back > 0-back contrast) in 269 healthy controls for the n-back task. Functional maps (thresholded at P < 0.001, t = 3.12, uncorrected) are projected on a rendered MNI template and shown from a lateral (Left) and top (Right) view for presentation purposes. Color bar represents t values. (B) Functional connectivity between right DLPFC seed and left HC during WM. Mean connectivity with right DLPFC seed for 269 healthy volunteers within left HC mask is shown in sagittal (Left) and axial (Right) sections, using an MNI structural template for presentation purposes. Color bar represents t values. Map coordinates refer to the standard space as defined by the MNI.
increasing attention within the schizophrenia research community, not least because of their importance in neurodevelopmental events important in circuit formation, such as neurite outgrowth and synaptogenesis, but also for their roles in synaptic signaling and neurotransmission processes linked to the pathophysiology of the disease (39, 43).

**NRCAM.** A notable gene in this category is the neuronal cell adhesion molecule (CAM) family of adhesion genes and is located in the middle of a genomic region strongly implicated in schizophrenia etiology and has been associated with schizophrenia in a Korean population (44, 45). Altered NRCAM levels have been reported in HC, DLPFC, and amygdala and in the cerebrospinal fluid of patients with schizophrenia (46–48). NRCAM polymorphisms have also been associated with variation in neurocognitive scores in patients with schizophrenia (49). As a group, CAM genes have also been associated with schizophrenia in a pathway analytical study (43). Transgenic mice lacking NRCAM isoforms show deficits in learning and long-term potentiation (LTP) in the hippocampus and in prepulse inhibition responses, whereas mice overexpressing the extracellular domain of NRCAM exhibit WM deficits and impaired plasticity in prefrontal regions (46, 50–53).

Of particular interest is the polysialated form of NRCAM (PSA-NRCAM), which is expressed specifically within inhibitory GABAergic interneurons. NRCAM-PSA—expressing interneurons in mice show reduced dendritic spine numbers, decreased arborization, and changes in the synaptic connectivity (54). A study of cultured hippocampal neurons showed that PSA-NRCAM is required for N-methyl-D-aspartate (NMDA) receptor-dependent LTP and acts as an antagonist at N2Rβ-subunit-bearing NMDA receptors, preventing glutamate-induced cell death (55, 56). The role of NRCAM at NMDA receptors is of particular interest given that NMDA receptor hypofunction in the prefrontal cortex is one of the leading schizophrenia hypotheses and has been linked to the cognitive symptoms and oscillatory disturbances associated with the disease (57). NMDA receptor antagonists have been found to produce schizophrenia-like symptoms in healthy subjects, disrupt WM function in rats, and impair WM performance when administered to the DLPFC in monkeys (58, 59).

**Neuroligin.** Another prominent adhesion gene in these categories is the NLGN1 gene. NLGN1 is a presynaptic adhesion protein with a critical role in synaptic formation. There is increasing interest in the neurexin family in schizophrenia because neurexins form transsynaptic complexes with schizophrenia-associated neurexin (NRX) proteins (61, 62). NLGN–NRX complexes are known to be important in brain development, and genetic variation in these genes has been associated with autism and schizophrenia (63–66). The NLGN1–NRX complex has been found to specifically localize to glutamatergic synapses, where it helps stabilize the synapse and recruit additional synaptic proteins involved in synapse structure and function. Several lines of evidence suggest an important role for NLGN1 in modulation of the NMDA-type glutamate receptor (61, 67, 68). In mice NLGN1 regulates the synaptic abundance of NMDA-type glutamate receptors (61). Overexpression of NLGN1 in mouse hippocampus results in increased inhibitory input and increases in glutamatergic neurotransmission (61, 68). This finding is in good agreement with the excitation–inhibition hypothesis of schizophrenia, which posits that the NMDA receptor in the cortex functions as a kind of “excitatory sensor” and that decreased NMDA activity, particularly within interneurons, can lead to alteration of systems-level neural dynamics (69).

Finally, there is evidence suggesting that neurexin genes impact a number of other genes and associated pathways linked to schizophrenia pathophysiology, including the neurexin receptor signaling compound and the discs, large homolog 4 (DLG4) molecule. However, further investigation of these downstream gene targets is needed to elucidate underlying neurobiological
CACHN2. Also part of the synapse organization and biogenesis gene set is the voltage-dependent calcium ion channel family member CACHN2. Voltage-gated calcium ion channels are distributed widely throughout the brain and are critical for mediating intracellular Ca\(^{2+}\) influx in response to action potentials at the synapse, and have an important role in NMDA receptor-independent synaptic plasticity processes (70). CACHN2 reached genome-wide significance in two large meta-analyses of schizophrenia GWAS, and also in bipolar disorder, which is known to share a considerable genetic overlap with schizophrenia (71–73). In addition to these specific observations, our findings agree with a growing body of genetic, structural, functional, and brain expression evidence suggesting an involvement of calcium-dependent regulatory processes in prefrontal–hippocampal network plasticity (74, 75) and the pathophysiology of schizophrenia (16, 76).

UBB. An interesting gene in this gene set is the ubiquitin gene. Ubiquitination processes have been closely linked to the assembly, connectivity, and function of the synapse, including the turnover of pre- and postsynaptic proteins. Abnormalities of ubiquitin expression have been reported in blood cells and the HC, PFC, and temporal gyrus of patients with schizophrenia (77–79). Gene sets related to the ubiquitin proteasome system have also been identified in two pathway analytical studies of schizophrenia, providing further evidence for the involvement of the ubiquitination processes in disease etiology (80). This is consistent with a growing number of studies suggesting that ubiquitin genes may function as upstream factors impacting the disturbed synaptic plasticity process reported in schizophrenia (81). Expression of genes of the ubiquitin proteasome system has been linked to positive symptom scores for schizophrenia and the dysregulation of the ubiquitin-proteasome system has been linked to psychosis in two independent samples (80).

GHRRL. Ghrelin is a metabolic peptide associated with the regulation of appetite and food intake; ghrelin levels in the brain have been found to change with atypical antipsychotic treatment (82). Interestingly, several lines of evidence have linked ghrelin to WM function (83). Infusion of ghrelin in rats to the HC and dentate gyrus regions in adult rats promoted synaptogenesis and was associated with enhanced spatial WM function (84). This is consistent with a study in healthy older subjects in which serum ghrelin was linked to poorer WM function (85). Ghrelin has also been associated with the synaptic accumulation of the AMPA glutamate receptors and increases in LTP, a form of synaptic plasticity that is thought to underlie learning and memory (86).

Limitations. Although the use of biologically based gene sets as units of analysis has several advantages, the GSEA of whole-genome neuroimaging data are not without limitations. First, although ontology-based gene sets offer the most complete catalogization of gene properties to date, we still possess a limited understanding of gene functions and pathways involved in normal and pathological brain function. Consequently, the gene sets used here will evolve over time as new information is incorporated, with potential effects on the reported findings.

Lastly, although our data are consistent with prior genetic association studies in schizophrenia and the neurobiology of plasticity-related neural functions, our inferences on the pathophysiology of schizophrenia rest on prior evidence linking the examined connectivity phenotype to disease risk and do not follow directly from our data.

Conclusion

A major challenge in schizophrenia research has been identifying and characterizing the complex neurobiological effects of multiple conjoined genetic variants contributing to disease risk. Leveraging the combined power of neuroimaging and GWAS, this study supports a role for genes involved in synapse organization and function in prefrontal–hippocampal interactions. Several genes in this pathway are highly consistent with the reported risk architecture of schizophrenia, and we provide independent evidence for the intermediate phenotype concept. In addition, we identified genes that are strongly implicated in the schizophrenia pathophysiology but have not been identified by classic genetic association studies. Our findings highlight the value of this biologically driven method in determining the mechanisms and genes underlying intermediate systems-level neural phenotypes linked to psychiatric disorders and should be generalized to other phenotypes and disorders of interest. We expect that a more pathway-oriented approach will also be helpful in linking up systems-level observations in humans with drug development in psychiatric disorders.

Materials and Methods

Subjects. We studied 269 healthy German volunteers with parents and grandparents of European origin from the population of the cities of Mannheim, Berlin, and Bönn [mean (±SD) age 29.3 ± 9.81 y, 135 female; details in Table S1]. Exclusion criteria included the presence of a lifetime history of psychiatric, neurological, or significant general medical illness, pregnancy, a history of head trauma and/or chronic alcohol or drug abuse. None of the volunteers had a first-degree relative with a psychiatric disorder or received psychotropic pharmacological treatment. All participants provided written, informed consent for a protocol approved by the Ethics Committee of the University of Heidelberg.

Genotyping. DNA was extracted from white blood cells according to standard methods, and subjects were genotyped using HumanHap 610 and 660w Quad BeadChips (Illumina Inc.). A standard quality control protocol was applied to the data: minor allele frequency >0.03, individual call rate >0.98 and genotype call rate >0.98, and Hardy-Weinberg equilibrium P > 0.001. This resulted in a total of 486,036 SNPs for subsequent association analysis with functional MRI (fMRI) data.

Image Acquisition. Whole-brain blood oxygenation level-dependent (BOLD) fMRI was performed on three identical 3-Tesla MRI scanners at the Central Institute of Mental Health in Mannheim and the medical faculties of the universities of Bonn and Berlin (Siemens Trio). All three sites used identical data acquisition protocols. Functional data were acquired using echo planar imaging sequences with the following specifications: 28 axial slices, 4-mm slice thickness, 1-mm gap, time to repetition/time to echo 2,000/30 ms, 80° flip angle, 192 mm × 192 mm field of view, and 64 × 64 matrix. To ensure comparable scanner magnet stability over time, quality assurance (QA) measurements were conducted following an established multicenter QA protocol at all sites (87).

fMRI WM Paradigm. DLPFC function and DLPFC–HC functional connectivity during WM was challenged with the n-back task, a well-established and reliable paradigm used extensively in imaging genetics (9, 29, 88). Briefly, subjects viewed a series of digits (1–4) presented sequentially for 500 ms and responded to a digit within an interval 1,000 ms before the current frame was highlighted to represent the target number to be maintained in memory. As the sequence progressed, the subject indicated which number corresponded to the highlighted number (via a button press) shown in the current frame (0-back, control condition) or two frames previously (2-back, experimental condition). The task is presented in eight blocks of 30 s, with alternating epochs of 0-back and 2-back conditions, giving a total run length of 4 min 15 s or 128 whole-brain scans. To allow task familiarization and to minimize performance effects in the scanner, participants were instructed during test versions of the paradigm offline before the scan.

Imaging Processing and Connectivity Analysis. Image processing and statistical analyses were conducted using statistical parametric mapping methods (SPM8; www.fil.ion.ucl.ac.uk/spm/software/spm8). Images were realigned to the first image, slice-time corrected, and spatially normalized into a standard stereotactic space [Montreal Neurological Institute (MMNI) template] with volume units (voxels) of 3 × 3 × 3 mm and smoothed with a 9-mm FWHM Gaussian filter. Our functional connectivity analysis used a seeded connectivity approach that closely follows a well-established approach for this risk phenotype previously used by our group and others (4, 10). First, individual first eigenvariate s of the seed time series were extracted from 6-mm spheres centered on the most significantly activated voxel in the 2-back > 0-back activation contrast in the right DLPFC (Brodmann areas 46 and lateral 9) as defined by the Wake Forest University PickAtlas (Fig. 1A provides an illustration of the 2-back > 0-back activation patterns at the...
group level). The use of functionally defined individual seed regions is thought to improve the sensitivity of the analysis to detect task-related effects and connectivity. To further the contribution of task-related coactivation to functional connectivity measures, we further adjusted the seed-time series for task-related variance. The time series of our first-level functional connectivity model were high-pass filtered (128 s) and adjusted for a global signal (first eigenvariate over the whole brain). To account for additional sources of variance, such as physiological noise and movement, first eigenvariates from anatomical masks of the cerebrospinal fluid and white matter were entered along with movement covariates into a whole-brain general linear model with the seed time-series as the covariance of interest.

Statistical Inference. We analyzed the association between SNPs and DLPCF–HC functional connectivity via the following steps. First-level p-images from our contrast of interest (mean functional connectivity with right DLPCF seed) were entered into a second-level multiple regression model in SPM, with age, sex, and site as covariates, and an additional four multidimensional scaling components to account for genetic stratification of the sample. To capture the individual subject’s strength of functional connectivity in a single parameter, we extracted the mean residual value within a standard anatomical left hippocampus mask (Wake Forest University PickAtlas) from this model. Finally, the association of SNPs with our single-parameter measure of DLPCF–HC functional connectivity was obtained by computing the number of minor alleles per individual and SNP (additive model) and performing a genome-wide linear association analysis in Plink (89), resulting in a corresponding p value for each SNP.

Mapping SNPs to Tissue. To map individual SNPs to genes and derive a gene-level summary score, we used the LDsnpR package, previously implemented by Erland et al. (26, 90). The LDsnpR package was used to assign SNPs to genes using chromosomal position and linkage disequilibrium information from Human Ensembl 66 release and the CEU (Utah residents with ancestry from northern and western Europe) sample from HapMap Phase II. SNPs were mapped to gene bins if they were (i) located within the specified gene boundary, (ii) within an additional 20-kb window to account for proximal regulatory elements, and (iii) if they were in linkage disequilibrium with another SNP that was located within the boundaries (pairwise linkage disequilibrium threshold ≤ 0.8) (21, 25, 91). Once SNPs were mapped to gene bins, the minimum SNP association p value per gene was extracted, and a gene score was produced using a modified Sidak test as implemented in LDsnpR (92). This method has been shown to successfully control any length bias that may arise from large genes that bear a considerable number of SNPs (90).

GSEA. GSEA tests whether particular biologically derived gene sets are over-represented among the loci that have been shown to be most associated with a phenotype (24). Here we used the adapted version of the weighted Kolmogorov-Smirnov statistic following published procedures (24). In brief, gene sets are sorted into a ranked list by association p values and are assigned to gene sets. For each gene set an enrichment score (ES) is then calculated by running down the ranked gene list, increasing an aggregate score when a gene from the gene set is met, and decreasing it if not. The ES is thus the maximum deviation from zero on the ranked list and reflects the degree to which a gene set correlates with the phenotype. The significance of each ES is estimated by an empirical permutation procedure, whereby gene association scores are shuffled across gene sets, and each ES is recalculated relative to this null distribution. The ES is then normalized to allow comparison of differently sized gene sets and to correct for the comparison of multiple gene sets using the FDR. Any gene set with an FDR q ≤ 0.05 was considered to be significant.

GSEA Software and Analysis Setup. All available gene sets were assigned to gene sets according to gene ontology category definitions provided by the Molecular Signatures Database (24). The gene ontology project categorizes genes into biologically related gene sets for three main domains (the molecular function, biological process, and cellular component ontologies) on the basis of experimental and computational evidence gleaned from bioinformatics databases (93). Given our a priori interest in the cellular and molecular events involved in schizophrenia, we assign genes to gene sets from the biological process gene ontology. To avoid overinflating enrichment scores by abnormally large or small gene sets, only gene sets with 15–200 genes were analyzed. This step generated 346 out of a total of 825 gene sets for further analysis by GSEA. To omit genes with weak evidence for involvement in a particular gene category, we further excluded any genes that were annotated to gene ontology categories on the basis of electronic evidence alone (i.e., uncurated comparison of sequence similarity). Using these gene sets we then performed a GSEA using freely available GSEA software developed by the Broad Institute (94).

ACKNOWLEDGMENTS. This study was supported by the German Federal Ministry of Education and Research (BMBF) through the Bernstein Center for Computational Neuroscience (BCCN) Heidelberg/Mannheim (Grant 01GQ1003B subproject C7 to A.M.-L.) and the Integrated Genome Research Network NGFNplus Moods (Grant 01GS08147 to A.M.-L. and M.R., Grant 01GS08144 to M.M.N., S.C. and H.W., and Grant 01GS08148 to A.H.). A.M.-L. received also funding through a National Alliance for Research on Schizophrenia and Depression Distinguished Investigator Award. H.T. receives grant support from the BMBF (Grant 01GQ1102).


See Retraction Published September 02, 2014

PNAS | July 1, 2014 | vol. 111 | no. 26 | 9661

Dixon et al.


