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Genome-Wide Significant Linkage of Schizophrenia-Related Neuroanatomical Trait to 12q24

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Abstract

The insula and medial prefrontal cortex (mPFC) share functional, histological, transcriptional and developmental characteristics and they serve higher cognitive functions of theoretical relevance to schizophrenia and related disorders. Meta-analyses and multivariate analysis of structural magnetic resonance imaging (MRI) scans indicate that gray matter density and volume reductions in schizophrenia are the most consistent and pronounced in a network primarily composed of the insula and mPFC. We used source-based morphometry, a multivariate technique optimized for structural MRI, in a large sample of randomly ascertained pedigrees (N = 887) to derive an insula-mPFC component and to investigate its genetic determinants. Firstly, we replicated the insula-mPFC gray matter component as an independent source of gray matter variation in the general

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population, and verified its relevance to schizophrenia in an independent case-control sample. Secondly, we showed that the neuroanatomical variation defined by this component is largely determined by additive genetic variation ($h^2 = 0.59$), and genome-wide linkage analysis resulted in a significant linkage peak at 12q24 ($LOD = 3.76$). This region has been of significant interest to psychiatric genetics as it contains the Darier's disease locus and other proposed susceptibility genes (e.g. *DAO*, *NOS1*), and it has been linked to affective disorders and schizophrenia in multiple populations. Thus, in conjunction with previous clinical studies, our data imply that one or more psychiatric risk variants at 12q24 are co-inherited with reductions in mPFC and insula gray matter concentration.

Keywords

Extended pedigrees; magnetic resonance imaging; insula; medial prefrontal cortex; quantitative trait locus

1. Introduction

Schizophrenia is a heritable disorder (Sullivan et al., 2003) but the genetic variation accounting for its inheritance is complex and difficult to characterize. Many genetic markers, both common and rare, are thought to contribute to genetic risk for schizophrenia and related disorders (Gratten et al., 2014). Efforts to localize susceptibility variants and investigate their downstream effects on protein synthesis and interactions are hindered by the heterogeneity and complexity of the clinical phenotype, and by the small effect sizes of the common variants typically identified by large-scale association studies. Family-based designs offer increased power to identify genetic variants, especially rare variants with potentially larger effect sizes (Williams and Blangero 1999). In addition, intermediate phenotypes that are heritable and genetically associated with a clinical diagnosis can facilitate variant localization, both because of their quantitative nature and because of their assumed proximity to the genetic effects (Glahn et al., 2007; Gottesman and Gould 2003). Simultaneously, these endophenotypes (Gottesman and Gould 2003) provide insights into the variants' influences on biological processes, yielding clues to pathological mechanisms that contribute to the expression of the clinical phenotype.

Neuroanatomical traits derived from magnetic resonance imaging (MRI) are logical endophenotypes as their selection can be informed by a large body of literature in clinical samples, and they are likely to be biologically intermediate between genes' functions and their more remote effects on behavioral phenotypes (Glahn et al., 2007). Despite marked clinical and methodological heterogeneity, multiple meta-analyses of voxel-based morphometry studies in schizophrenia have identified the insula and the medial prefrontal cortex (mPFC) as the neuroanatomical regions most consistently associated with schizophrenia across all published case-control studies (Bora et al., 2011; Fornito et al., 2009; Glahn et al., 2008; Palaniyappan et al., 2012; Shepherd et al., 2012).

Source-based morphometry (SBM) (Xu et al., 2009) is a multivariate method that decomposes gray matter concentration images, derived from T_1 -weighted MRI scans, into spatially independent sources. The outcome is a matrix of weights for each individual on

each source map, which can be used as dependent variables instead of voxel-wise values. As such, SBM dramatically reduces the number of comparisons typically performed in voxel-based analyses. SBM also addresses other common problems in MRI analysis, including the choice of smoothness kernel and the non-stationarity of image smoothness (Hayasaka and Nichols 2003). Remarkably, the application of SBM has repeatedly identified a single component comprising the mPFC and the insula – the same regions as the case-control meta-analyses (15-19) – as the most affected anatomical network in schizophrenia patients (Gupta et al., In Press; Kasperek et al., 2010; Turner et al., 2012; Xu et al., 2009). Thus, following an endophenotype strategy, quantification and localization of the genetic influences on this schizophrenia-associated neuroanatomical trait could provide testable candidate genes and generate novel hypotheses about mechanisms underlying susceptibility for schizophrenia.

Here, we used SBM in a large sample of randomly ascertained pedigrees. Our aims were threefold: (1) to replicate the schizophrenia-associated insula-mPFC source as a spatially independent component in a new sample representative of the general population; (2) to estimate its heritability; and (3) to localize this genetic influence to specific genomic regions using linkage analysis.

2. Materials and Methods

2.1 GOBS extended pedigree sample

Participants were individuals of Mexican American ancestry who took part in the Genetics Of Brain Structure and Function Study (GOBS) (McKay et al., 2014; Olvera et al., 2011), which is an extension of the San Antonio Family study (Mitchell et al., 1996). Individuals were randomly selected from the community with the only constraints that they were part of a large family of Mexican-American ancestry and lived within the San Antonio region. For the present analysis, subjects were excluded for MRI contraindications, documented medical history of neurological illness, or any neurological event visible on the T₁-weighted scans (see section 2.3). Of the participants in the final analysis, 22 self-reported history of a neurological event or illness (18 stroke, 1 Parkinson's disease, 3 multiple sclerosis, 1 brain surgery), but excluding these individuals did not change the pattern of results presented. After quality control procedures (see below) T₁-weighted scans were available for 887 individuals (532 female), from 69 pedigrees ranging from 2 to 90 family members, and 46 singletons. Participants were between 18 and 85 years old (mean= 44; standard deviation(SD)=15).

History of axis-I disorders was assessed using the Mini International Neuropsychiatric Interview (Sheehan et al., 1998). Of the individuals included in the main analysis, four had a diagnosis of schizophrenia, fifteen of bipolar disorder, and four of schizoaffective disorder. For our main results, we performed additional analyses excluding these individuals (see results section). Additionally, 297 participants had a history of major depression, and 118 had a history of an anxiety disorder, and we performed additional analyses co-varying for these diagnoses.

2.2 MR imaging and processing in GOBS

An MRI protocol optimized for cortical gray matter measurements (Kochunov and Davis 2009), with a retrospective motion correction technique (Kochunov et al., 2006), was used. For each participant, seven T₁-weighted scans were obtained in a Siemens 3 Tesla Trio scanner located at the Research Imaging Institute, University of Texas Health Science Center, using a magnetization prepared sequence with an adiabatic inversion contrast-forming pulse (scan parameters: TE/TR/TI=3.04/2100/785 ms, flip angle=11 degrees). As in (Kochunov et al., 2006), for each subject the seven volumes were coregistered and averaged.

Upon visual inspection, five individuals were excluded for neurological abnormalities, and one for a scanner artifact. The resulting images were further processed in SPM5 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm5/>), using the same parameters as in Turner et al. (Gupta et al., In Press; Turner et al., 2012) and Xu et al. (Xu et al., 2009). Gray matter maps were nonlinearly normalized, resliced to a 2mm³ MNI template and segmented into gray matter, white matter and cerebrospinal fluid (Ashburner and Friston 2000). The accuracy of the segmentations and normalizations was ensured by visual inspection and by calculating correlations with the average normalized gray matter map for the entire sample. Five individuals were excluded because of segmentation or normalization problems.

2.3 Source-based morphometry

SBM (Kasperek et al. 2010, ; Xu et al., 2009) (<http://mialab.mrn.org/software/gift/>) is a multivariate method that decomposes structural images into spatially distinct sources using independent component analysis (Bell and Sejnowski 1995). The decomposition of the subject-by-voxel matrix (X) results in a subject-by-component mixing matrix (W), which contains the weights of the subjects on each component; and a component-by-voxel source matrix (C), which contains the loadings of each voxel for each component. This decomposition¹ can be noted as follows (Calhoun et al., 2001):

$$X=W^*C$$

And therefore:

$$W=X^*C^{-1}$$

For a subject i , the weight of a component j reflects the overall gray matter concentration for that component map of n voxels. More quantitatively, the weight can be conceived of as the sum of each voxel's observed gray matter value multiplied by each voxel's loading on the component:

$$W_{i,j} = \sum_{k=1}^n X_{i,k} C_{k,j}^{-1}$$

¹In practice this decomposition of interest is preceded by an initial principal component analysis step on the subject-by-voxel matrix X to obtain a square matrix RX, as explained in Calhoun et al. (32). For clarity we have omitted this step from the current explanation and also note the inverse of C as C^{-1} even though it would technically be non-square if we omitted the PCA step.

These weights can be used as dependent variables in subsequent analyses.

The optimal number of components for our data was estimated according to an information criteria algorithm (Li et al., 2007). To determine the stability of the decomposition, ICASSO (Himberg et al., 2004) was used, with random value initiation and bootstrapping options, for 20 repetitions.² The stability indices for all components were higher than 0.97.

2.4 Relevance to schizophrenia: application to an independent case-control cohort

While we had strong *a priori* evidence for the involvement of insula and mPFC gray matter in schizophrenia (Bora et al., 2011; Fornito et al., 2009; Glahn et al., 2008; Palaniyappan et al., 2012; Shepherd et al., 2012; Turner et al., 2012), we also directly verified the relevance of the currently derived component to brain morphology of schizophrenia in a separate dataset of 936 healthy control participants (HC) and 784 patients with schizophrenia (SCZ), aggregated from 8 independent studies. More detailed information about the case-control sample are presented in the Supplementary Materials and in Gupta et al. (Gupta et al., In Press).

Using spatial-temporal regression, a method available in the SBM toolbox, we obtained weighting scores for each individual in the case-control dataset for the components identified using the GOBS data. By entering these as the dependent variable in a regression with study site, diagnosis and their interactions as factors, we tested directly whether gray matter concentration defined by the component extracted from the GOBS dataset was reduced in patients with schizophrenia.

In addition, we quantified the similarity between the components derived from the GOBS and the case-control datasets by calculating (1) pairwise correlations of the voxel loading values across maps from both datasets; and (2) Dice coefficients of the thresholded maps at $z > 3$, where the Dice coefficient is defined as twice the number of voxels with the same value in both maps divided by total number of voxels (within the masks).

2.5 Heritability analysis

All quantitative genetics analyses were performed in SOLAR (Almasy and Blangero 1998), which decomposes the variance of a trait into genetic and environmental components by modeling the covariance between individuals as a function of their genetic proximity. Typically, the trait variance is decomposed into an additive genetic effects (heritability), covariate effects (sex, age, age², age × sex, age² × sex), and residual environmental effects. We also tested for cubic effects of age, but these were negligible ($p > 0.9$) and dropped from the model. The significance of each variance component is assessed by a likelihood-ratio test comparing the final model to the model without the variable of interest. Using the subject's weights on the insula-mPFC component as a trait, this method yielded an index of the overall heritability (h^2) of gray matter concentration within the anatomical regions of the component. Prior to running SOLAR, eight outliers (mean ± 3*SD) were removed from the

²Note that in this case bootstrapping is applied to estimate the degree of variability across iterations, and not – as commonly is the case – to estimate a null-distribution, which would require many more iterations.

data and the remaining weights were transformed using an inverse normalization transformation.

2.6 Linkage and association analyses

Linkage analysis was performed in SOLAR, by adding location-specific identity-by-descent (IBD) information to the above heritability model. IBD means that two individuals within the same pedigree not only share the same genotype, but also inherited it from the same founder. Here, for the GOBS pedigrees the IBD matrices were estimated as in Curren et al. (2013), using the Loki package (Heath et al., 1997). In brief, for 15,000 SNPs³ across the genome, which were selected to be in linkage equilibrium ($r < 0.2$), Loki applies Markov Chain Monte Carlo sampling methods to empirically estimate the pairwise IBD probabilities for each SNP between each individual (Heath et al., 1997).

The significance of the contribution of each locus is quantified by a LOD score, defined as the logarithm (base 10) of the ratio of the likelihood of the model with the locus-specific IBD matrix to the model without this component (i.e. the same model used to test heritability). The LOD threshold for genome-wide significance was determined a priori for the complex pedigree structure of GOBS. This calculation is based on Gaussian models of the probability of crossover rates under the null distribution (Feingold et al., 1993), given our pedigree structure, the number of SNPs in our IBD matrix and known Haldane maps. Given our pedigree structure and distribution of markers, a LOD of 2.9 is required for genome-wide significant linkage (genome-wide $\alpha < 0.05$).

To further determine whether any specific SNPs were driving the observed linkage, association analysis was performed for all common SNPs under the linkage peak (defined as all consecutive loci with LODs greater than half the maximum LOD). DNA was extracted from lymphocytes, genotyped using Illumina beadchips (Human1M-Duo Beadchip; or HumanHap550 BeadChip in tandem with HumanHap450S Beadchip), and checked for accordance with Mendelian consistency as described previously (Sprooten et al., 2014). To account for pedigree structure, association analysis was performed in SOLAR, using methods identical to (Sprooten et al., 2014). In brief, the minor allele dosage of each tagging SNP is added as a covariate to the model used for heritability. A corrected p-value was calculated according to family-wise error rate at 5%, by calculating in SOLAR the effective number of independent SNPs under the peak, adjusted for linkage disequilibrium (LD) (Moskvina and Schmidt 2008).

3. Results

3.1 Identification and validation of insula-mPFC component

The method of Li (Li et al., 2007) estimated that the GOBS gray matter images were optimally explained by 21 independent sources.

³Note that here the SNPs are not used to test for association testing, but merely to represent an independent locus for IBD estimation to perform linkage.

As hypothesized, one component (Figure 1) closely resembled the insula-mPFC clusters resulting from the aforementioned voxel-based morphometry meta-analyses (Bora et al., 2011 ; Palaniyappan et al., 2012; Shepherd et al., 2012), as well as previous SBM studies (Gupta et al., In Press; Turner et al., 2012; Xu et al., 2009). The bilateral insular parts of this component contained voxels in the insula and temporal pole, extending to the inferior frontal, orbitofrontal, opercular and superior temporal gyri. The frontal cluster of the component contained voxels in the anterior cingulate and paracingulate gyrus, frontal pole, medial frontal cortex and superior frontal gyrus. A small number of voxels were negatively correlated with the mPFC and insula, mostly containing white matter in the superior parietal lobes and splenium.

In line with qualitative comparisons to the literature, spatio-temporal regression in the case-control dataset revealed that the weights on this insula-mPFC component were highly significantly different between schizophrenia patients and healthy participants ($F = 292.69$, $p = 1.19 \times 10^{-60}$), in the absence of site-by-diagnosis interactions ($F=1.24$, $p=0.22$). The correlation coefficient of the loadings across voxels between this map and the map derived from the case-control data was 0.58 and the Dice coefficient was 0.97, both indicating a high degree of overlap.

The present paper focuses on the insula-mPFC cluster. We provide information about all 21 component maps including heritability estimates and case-control statistics in the Supplementary Materials.

3.2 Quantitative trait localization

The polygenic model in SOLAR using the weights of the insula-mPFC component as quantitative trait revealed that gray matter in this region is significantly heritable ($h^2=0.59$; $p=1.78 \times 10^{-15}$). Linkage analysis resulted in a genome-wide significant peak on chromosome 12 at 12q24 (12q24.11-12q24.23; maximum LOD=3.76; Figure 2).

The linkage peak contained 392 tagging SNPs. Taking into account LD, the peak-wide corrected p-value for SNP associations was 1.64×10^{-4} . None of the SNPs were peak-wide significant (Supplementary Figure 2). The strongest association ($p=7.71 \times 10^{-4}$) was found for rs7133582, an intronic SNP in a transcription factor binding site in *KSR2*. Several other nearby SNPs had modest to strong associations with the insula-mPFC trait, altogether spanning 12q24.21 to 12q24.23.

Pedigree-specific LODs were all <1 , indicating that linkage was not driven by any specific pedigree. The heritability and the linkage results remained similar when excluding individuals with self-reported history of neurological events or illness, schizophrenia, schizoaffective disorder and bipolar disorder ($h^2=0.52$; LOD=3.19); and when co-varying for history of anxiety disorders ($h^2 = 0.60$; LOD=3.06) and major depressive disorder ($h^2=0.60$; LOD=2.97). There were no significant effects of history of major depression ($p > 0.8$) or anxiety disorders ($p>0.3$) on the component's weights.

To further assess the value of the SBM approach, we also extracted gray matter concentration averages within a binarized mask of the component map ($z > 3$) for each

individual. This univariate phenotype was less heritable ($h^2=0.47$, $p=3.08 \times 10^{-11}$), and the maximum LOD score on chromosome 12 was found at the same marker at 12q24 as the multivariate phenotype, but was much lower (LOD = 1.82).

4. Discussion

We used multivariate analyses of MRI images to extract a gray matter component comprising the insula and the mPFC, which have previously been shown to be the most pronounced (Gupta et al., In Press; Turner et al., 2012) and most consistently implicated gray matter regions in schizophrenia (Bora et al., 2011; Fornito et al., 2009; Glahn et al., ; Palaniyappan et al., 2012; Shepherd et al., 2012). We directly confirmed that gray matter defined by our empirically derived component was reduced in patients with schizophrenia from an independent case-control sample. Next, we found that the overall gray matter concentration in this component is heritable, and following genome-wide linkage analysis, we identified a quantitative trait locus for this component at 12q24.

4.1 Replication and interpretation of insula-mPFC component

So far, SBM has been predominantly applied to case-control studies in schizophrenia (Gupta et al., In Press; Turner et al., ; Xu et al., 2009). Together these studies investigated three independent samples, in four separate analyses, all of which highlight the insula-mPFC component as the most important gray matter component in association with schizophrenia. The replication and heritability of this component in a sample representative of the general population supports the reliability of this technique, and indicates that gray-matter variation in this network is influenced by genetic factors.

The notion of the mPFC-insula as a coherent network is consistent with histological, anatomical, functional and developmental similarities between these regions. The insula and the mPFC display similarly high rates of cortical thickening during neonatal development (Lyall et al., 2014) and throughout childhood (Sowell et al., 2004). Histologically they are also similar, most notably because of the distinct and exclusive presence of von Economo neurons in these regions (Butti et al., 2013). As key regions of the limbic system, and more specifically the “salience network” (Seeley et al., 2007), the mPFC and insula interact intensely to serve higher-order cognitive processes such as social and self-awareness, intuition, error monitoring and interoception. As such, their dysfunction has been postulated to lie at the core of the experience of psychotic symptoms (Kapur 2003; Palaniyappan et al., 2012; Pu et al., 2012). In a complementary theory, interactions between superior temporal, inferior frontal regions and the mPFC have long been hypothesized as key to the experience of auditory verbal hallucinations (Fletcher et al., 1999; Stephan et al., 2009).

4.2 Locus 12q24

We identified a 10Mb region at 12q24 linked to the insula-mPFC gray matter phenotype. This region has been of great interest to psychiatric genetics since Craddock et al. (Craddock et al., 1994) reported a cosegregation of bipolar disorder and Darier’s disease, a skin disease that is caused by mutations in *ATP2A2* (Bashir et al., 1993; Craddock et al., 1993) (Supplementary Figure 2). Subsequently, dozens of studies reported linkage of 12q24 to

affective disorders in the UK, Ireland, Germany, Denmark, Canada, Iceland and Finland in locations spanning from 12q22 to 12q24 (Ekholm et al., 2003; Ewald et al., 2003; Jones et al., 2002; McInnis et al., ; Morissette et al., 1999). Fewer but better powered studies have linked schizophrenia to 12q24 with loci mostly concentrated in 12q24.11-12q24.31 (113-128 Mb; Assembly GRCh37/hg19) (Bulayeva et al., 2007; DeLisi et al., 2002; Faraone et al., 2006; Holmans et al., 2009; Moises et al., 1995; Williams et al., 2003). This narrowed region matches our mPFC-insula locus, as well as another linkage result in relation to neurocognitive performance in schizophrenia patients (Lien et al., 2010).

In our association study we were unable to localize our linkage signal to any specific variants. The strongest association was found for rs7133582, a SNP in a transcription factor binding site of *KSR2*, a functionally poorly characterized gene that is involved in the MAPK and ERK signaling pathways (Dougherty et al., 2009). Similarly, despite the considerable interest in the region, previous studies have not been able to unequivocally identify specific genes that may drive linkage results at 12q24. Although in the most recent genome-wide association analysis of schizophrenia the Psychiatric Genomics Consortium identified a top SNP in the Darier's gene *ATP2A2* with $p < 10^{-9}$ (Schizophrenia Working Group of the Psychiatric Genomics 2014), in the original Darier's disease pedigrees risk for psychiatric disorders did not map to *ATP2A2* itself (Jacobsen et al., 2001) and the co-segregation of psychiatric symptoms with Darier's disease was thought to be due to nearby variation in LD (Jones et al., 2002). Other efforts to identify specific neuropsychiatric risk genes at 12q24 overall yielded inconclusive results (Dawson et al., 1995; Green et al., 2005; Green et al., 2009; Jacobsen et al., 1996; Shink et al., 2005). Several large-scale family studies highlight the genetic heterogeneity at 12q24 (Bulayeva et al., 2007; McInnis et al., 2003; Shink et al., 2005), and it has been suggested that the wider 12q23-12q24 region contains multiple genes that may influence neuropsychiatric phenotypes (Barden et al., 2006; Shink et al., 2005). The original fine-mapping studies suggested *CUX2*, *FAM109A* (or FLJ32356) (Glaser et al., 2005), *P2RX7*, *CAMKK2*, (Barden et al., 2006), and *LINC00944* ("Slynar gene") (Buttenschon et al., 2010; Kalsi et al., 2006). However 12q24 also contains the schizophrenia candidate genes *DAO* (Verrall et al., 2010) and *NOS1* (Cui et al., ; Silberberg et al., 2010; Wockner et al., 2014). Interestingly, during fetal development *NOS1* is transiently highly expressed in the mPFC and insula only (Funk and Kwan 2014), and variants in *NOS1* have been associated with prefrontal morphology and function (Rose et al., 2012). Finally, rs7294919, the genome-wide association with hippocampal volume identified by the ENIGMA and CHARGE consortia (Bis et al., 2012; Stein et al., 2012) also lies under our peak.

4.3 Strengths and limitations

An advantage of our multivariate voxel-based analysis is that it gave rise to a reliably identifiable and data-driven single trait that was known to be relevant to schizophrenia based on a large body of pre-existing literature. However, a limitation of all voxel-based techniques is that the concentration values bear an indirect relationship to the physiological, morphological and cellular properties within each voxel. Both regional thickness and surface area contribute independently to voxel-wise gray matter concentrations (Rimol et al., 2012; Winkler et al., 2010), and likely local cortical curvature and white matter morphology also

play a role. As such, an understanding of the biological mechanisms of the genetic effects we identified requires further specification of the morphological properties that contribute to the schizophrenia-related phenotype we investigated here.

Secondly, there is a multitude of *a priori* evidence and a strong rationale for the relevance of our quantitative trait to schizophrenia, which we also directly confirmed in an independent case-control dataset. However, a limitation of our study, as of most intermediate phenotype studies, is that neither endophenotype-disease associations nor quantitative genetics analyses directly test the assumption that the endophenotype lies on the *causal* pathway from genetic risk variants to development of disease. This is a general limitation of the field that can only truly be addressed by longitudinal designs, which are difficult to obtain to the same quantity as the data we present here, and to this date lack the statistical power and/or suitable pedigree structures for gene localization and identification.

Thirdly, while our linkage peak was not driven by any specific families within our sample, the detection of a genetic signal that is potentially obscured by locus heterogeneity (whether caused by rare or common variants) is likely facilitated by the use of a quantitative intermediate phenotype and the recruitment of large pedigrees. However, as discussed, we were unable to localize the genetic effects to any specific genes or variants. Large-scale family-based analysis of deep sequence data may be necessary to obtain more definitive answers regarding which and to what extent specific genes at 12q24 influence brain morphology and neuropsychiatric phenotypes.

4.4 Conclusions

There is compelling evidence that gray matter concentration in the insula and mPFC is reduced in patients with schizophrenia and in their unaffected family members. The genomic region 12q24 has been linked to psychiatric disorders in multiple populations worldwide and contains many genes of interest for neuropsychiatric phenotypes. Our findings indicate that genetic variation in this region also contributes to gray matter concentration in the insula and mPFC in the general population. Thus, mPFC and insula morphology are likely neuroanatomical correlates of schizophrenia that are co-inherited with schizophrenia susceptibility variants at 12q24.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. *American journal of human genetics*. 1998; 62(5):1198–1211. [PubMed: 9545414]
- Ashburner J, Friston KJ. Voxel-based morphometry--the methods. *NeuroImage*. 2000; 11(6 Pt 1):805–821. [PubMed: 10860804]
- Barden N, Harvey M, Gagne B, Shink E, Tremblay M, Raymond C, Labbe M, Villeneuve A, Rochette D, Bordeleau L, Stadler H, Holsboer F, Muller-Myhsok B. Analysis of single nucleotide polymorphisms in genes in the chromosome 12Q24.31 region points to P2RX7 as a susceptibility gene to bipolar affective disorder. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*. 2006; 141B(4):374–382.
- Bashir R, Munro CS, Mason S, Stephenson A, Rees JL, Strachan T. Localisation of a gene for Darier's disease. *Human molecular genetics*. 1993; 2(11):1937–1939. [PubMed: 7506604]
- Bell AJ, Sejnowski TJ. An information-maximization approach to blind separation and blind deconvolution. *Neural computation*. 1995; 7(6):1129–1159. [PubMed: 7584893]
- Bis JC, DeCarli C, Smith AV, van der Lijn F, Crivello F, Fornage M, Debette S, Shulman JM, Schmidt H, Srikanth V, Schuur M, Yu L, Choi SH, Sigurdsson S, Verhaaren BF, DeStefano AL, Lambert JC, Jack CR Jr, Struchalin M, Stankovich J, Ibrahim-Verbaas CA, Fleischman D, Zijdenbos A, den Heijer T, Mazoyer B, Coker LH, Enzinger C, Danoy P, Amin N, Arfanakis K, van Buchem MA, de Bruijn RF, Beiser A, Dufouil C, Huang J, Cavalieri M, Thomson R, Niessen WJ, Chibnik LB, Gislason GK, Hofman A, Pikula A, Amouyel P, Freeman KB, Phan TG, Oostra BA, Stein JL, Medland SE, Vasquez AA, Hibar DP, Wright MJ, Franke B, Martin NG, Thompson PM, Enhancing Neuro Imaging Genetics through Meta-Analysis C, Nalls MA, Uitterlinden AG, Au R, Elbaz A, Beare RJ, van Swieten JC, Lopez OL, Harris TB, Chouraki V, Breteler MM, De Jager PL, Becker JT, Vernooij MW, Knopman D, Fazekas F, Wolf PA, van der Lugt A, Gudnason V, Longstreth WT Jr, Brown MA, Bennett DA, van Duijn CM, Mosley TH, Schmidt R, Tzourio C, Launer LJ, Ikram MA, Seshadri S, Cohorts for H; Aging Research in Genomic Epidemiology C. Common variants at 12q14 and 12q24 are associated with hippocampal volume. *Nature genetics*. 2012; 44(5):545–551. [PubMed: 22504421]
- Bora E, Fornito A, Radua J, Walterfang M, Seal M, Wood SJ, Yucel M, Velakoulis D, Pantelis C. Neuroanatomical abnormalities in schizophrenia: a multimodal voxelwise meta-analysis and meta-regression analysis. *Schizophrenia research*. 2011; 127(1-3):46–57. [PubMed: 21300524]
- Bulayeva KB, Glatt SJ, Bulayev OA, Pavlova TA, Tsuang MT. Genome-wide linkage scan of schizophrenia: a cross-isolate study. *Genomics*. 2007; 89(2):167–177. [PubMed: 17140763]
- Buttenschoon HN, Foldager L, Flint TJ, Olsen IM, Deleuran T, Nyegaard M, Hansen MM, Kallunki P, Christensen KV, Blackwood DH, Muir WJ, Straarup SE, Als TD, Nordentoft M, Borglum AD, Mors O. Support for a bipolar affective disorder susceptibility locus on chromosome 12q24.3. *Psychiatric genetics*. 2010; 20(3):93–101. [PubMed: 20410851]
- Butti C, Santos M, Uppal N, Hof PR. Von Economo neurons: clinical and evolutionary perspectives. *Cortex; a journal devoted to the study of the nervous system and behavior*. 2013; 49(1):312–326.
- Calhoun VD, Adali T, Pearlson GD, Pekar JJ. Spatial and temporal independent component analysis of functional MRI data containing a pair of task-related waveforms. *Human brain mapping*. 2001; 13(1):43–53. [PubMed: 11284046]
- Craddock N, Dawson E, Burge S, Parfitt L, Mant B, Roberts Q, Daniels J, Gill M, McGuffin P, Powell J, et al. The gene for Darier's disease maps to chromosome 12q23-q24.1. *Human molecular genetics*. 1993; 2(11):1941–1943. [PubMed: 8129825]
- Craddock N, Owen M, Burge S, Kurian B, Thomas P, McGuffin P. Familial cosegregation of major affective disorder and Darier's disease (keratosis follicularis). *The British journal of psychiatry : the journal of mental science*. 1994; 164(3):355–358. [PubMed: 8199789]
- Cui H, Nishiguchi N, Yanagi M, Fukutake M, Mouri K, Kitamura N, Hashimoto T, Shirakawa O, Hishimoto A. A putative cis-acting polymorphism in the NOS1 gene is associated with

schizophrenia and NOS1 immunoreactivity in the postmortem brain. *Schizophrenia research*. 2010; 121(1-3):172–178. [PubMed: 20605417]

Curran JE, McKay DR, Winkler AM, Olvera RL, Carless MA, Dyer TD, Kent JW Jr, Kochunov P, Sprooten E, Knowles EE, Comuzzie AG, Fox PT, Almasy L, Duggirala R, Blangero J, Glahn DC. Identification of pleiotropic genetic effects on obesity and brain anatomy. *Human heredity*. 2013; 75(2-4):136–143. [PubMed: 24081229]

Dawson E, Gill M, Curtis D, Castle D, Hunt N, Murray R, Powell J. Genetic association between alleles of pancreatic phospholipase A2 gene and bipolar affective disorder. *Psychiatric genetics*. 1995; 5(4):177–180. [PubMed: 8750360]

DeLisi LE, Shaw SH, Crow TJ, Shields G, Smith AB, Larach VW, Wellman N, Loftus J, Nanthakumar B, Razi K, Stewart J, Comazzi M, Vita A, Heffner T, Sherrington R. A genome-wide scan for linkage to chromosomal regions in 382 sibling pairs with schizophrenia or schizoaffective disorder. *The American journal of psychiatry*. 2002; 159(5):803–812. [PubMed: 11986135]

Dougherty MK, Ritt DA, Zhou M, Specht SI, Monson DM, Veenstra TD, Morrison DK. KSR2 is a calcineurin substrate that promotes ERK cascade activation in response to calcium signals. *Molecular cell*. 2009; 34(6):652–662. [PubMed: 19560418]

Ekholm JM, Kieseppa T, Hiekkalinna T, Partonen T, Paunio T, Perola M, Ekelund J, Lonnqvist J, Pekkarinen-Ijas P, Peltonen L. Evidence of susceptibility loci on 4q32 and 16p12 for bipolar disorder. *Human molecular genetics*. 2003; 12(15):1907–1915. [PubMed: 12874110]

Ewald H, Kruse TA, Mors O. Genome wide scan using homozygosity mapping and linkage analyses of a single pedigree with affective disorder suggests oligogenic inheritance. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*. 2003; 120B(1):63–71.

Faraone SV, Hwu HG, Liu CM, Chen WJ, Tsuang MM, Liu SK, Shieh MH, Hwang TJ, Ou-Yang WC, Chen CY, Chen CC, Lin JJ, Chou FH, Chueh CM, Liu WM, Hall MH, Su J, Van Eerdewegh P, Tsuang MT. Genome scan of Han Chinese schizophrenia families from Taiwan: confirmation of linkage to 10q22.3. *The American journal of psychiatry*. 2006; 163(10):1760–1766. [PubMed: 17012687]

Feingold E, Brown PO, Siegmund D. Gaussian models for genetic linkage analysis using complete high-resolution maps of identity by descent. *American journal of human genetics*. 1993; 53(1):234–251. [PubMed: 8317489]

Fletcher P, McKenna PJ, Friston KJ, Frith CD, Dolan RJ. Abnormal cingulate modulation of fronto-temporal connectivity in schizophrenia. *NeuroImage*. 1999; 9(3):337–342. [PubMed: 10075903]

Fornito A, Yucel M, Patti J, Wood SJ, Pantelis C. Mapping grey matter reductions in schizophrenia: an anatomical likelihood estimation analysis of voxel-based morphometry studies. *Schizophrenia research*. 2009; 108(1-3):104–113. [PubMed: 19157788]

Funk OH, Kwan KY. Nitric oxide signaling in the development and evolution of language and cognitive circuits. *Neuroscience research*. 2014

Glahn DC, Laird AR, Ellison-Wright I, Thelen SM, Robinson JL, Lancaster JL, Bullmore E, Fox PT. Meta-analysis of gray matter anomalies in schizophrenia: application of anatomic likelihood estimation and network analysis. *Biological psychiatry*. 2008; 64(9):774–781. [PubMed: 18486104]

Glahn DC, Thompson PM, Blangero J. Neuroimaging endophenotypes: strategies for finding genes influencing brain structure and function. *Human brain mapping*. 2007; 28(6):488–501. [PubMed: 17440953]

Glaser B, Kirov G, Green E, Craddock N, Owen MJ. Linkage disequilibrium mapping of bipolar affective disorder at 12q23-q24 provides evidence for association at CUX2 and FLJ32356. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*. 2005; 132B(1):38–45.

Gottesman II, Gould TD. The endophenotype concept in psychiatry: etymology and strategic intentions. *The American journal of psychiatry*. 2003; 160(4):636–645. [PubMed: 12668349]

Gratten J, Wray NR, Keller MC, Visscher PM. Large-scale genomics unveils the genetic architecture of psychiatric disorders. *Nature neuroscience*. 2014; 17(6):782–790. [PubMed: 24866044]

- Green E, Elvidge G, Jacobsen N, Glaser B, Jones I, O'Donovan MC, Kirov G, Owen MJ, Craddock N. Localization of bipolar susceptibility locus by molecular genetic analysis of the chromosome 12q23-q24 region in two pedigrees with bipolar disorder and Darier's disease. *The American journal of psychiatry*. 2005; 162(1):35–42. [PubMed: 15625199]
- Green EK, Grozeva D, Raybould R, Elvidge G, Macgregor S, Craig I, Farmer A, McGuffin P, Forty L, Jones L, Jones I, O'Donovan MC, Owen MJ, Kirov G, Craddock N. P2RX7: A bipolar and unipolar disorder candidate susceptibility gene? *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*. 2009; 150B(8):1063–1069.
- Gupta CN, Calhoun VD, Rachkova S, Chen J, Liu J, Segall J, Franke B, Zwiers M, Aias-Vasquez A, Buitelaar J, Fischer S, Fernandez G, van Erp TGM, Potkin S, Ford J, Mathalon D, McEwen S, Lee JH, Mueller BA, Greve DN, Andreassen O, Agartz I, Gollub RL, Sponheim SR, Ehrlich S, Wang L, Pearlson G, Glahn DC, Sprooten E, Mayer AR, Stephen J, Jung RE, Canive J, Bustillo J, Turner JA. Patterns of gray matter abnormalities in schizophrenia based on an international mega-analysis. *Schizophrenia bulletin*. In Press.
- Heath SC. Markov Chain Segregation and Linkage Analysis for Oligogenic Models. *Am J Human Genet*. 1997; 61:748–760. [PubMed: 9326339]
- Hayasaka S, Nichols TE. Validating cluster size inference: random field and permutation methods. *NeuroImage*. 2003; 20(4):2343–2356. [PubMed: 14683734]
- Himberg J, Hyvarinen A, Esposito F. Validating the independent components of neuroimaging time series via clustering and visualization. *NeuroImage*. 2004; 22(3):1214–1222. [PubMed: 15219593]
- Holmans PA, Riley B, Pulver AE, Owen MJ, Wildenauer DB, Gejman PV, Mowry BJ, Laurent C, Kendler KS, Nestadt G, Williams NM, Schwab SG, Sanders AR, Nertney D, Mallet J, Wormley B, Lasseter VK, O'Donovan MC, Duan J, Albus M, Alexander M, Godard S, Ribble R, Liang KY, Norton N, Maier W, Papadimitriou G, Walsh D, Jay M, O'Neill A, Lerer FB, Dikeos D, Crowe RR, Silverman JM, Levinson DF. Genomewide linkage scan of schizophrenia in a large multicenter pedigree sample using single nucleotide polymorphisms. *Molecular psychiatry*. 2009; 14(8):786–795. [PubMed: 19223858]
- Jacobsen N, Daniels J, Moorhead S, Harrison D, Feldman E, McGuffin P, Owen MJ, Craddock N. Association study of bipolar disorder at the phospholipase A2 gene (PLA2A) in the Darier's disease (DAR) region of chromosome 12q23-q24.1. *Psychiatric genetics*. 1996; 6(4):195–199. [PubMed: 9149325]
- Jacobsen NJ, Franks EK, Elvidge G, Jones I, McCandless F, O'Donovan MC, Owen MJ, Craddock N. Exclusion of the Darier's disease gene, ATP2A2, as a common susceptibility gene for bipolar disorder. *Molecular psychiatry*. 2001; 6(1):92–97. [PubMed: 11244492]
- Jones I, Jacobsen N, Green EK, Elvidge GP, Owen MJ, Craddock N. Evidence for familial cosegregation of major affective disorder and genetic markers flanking the gene for Darier's disease. *Molecular psychiatry*. 2002; 7(4):424–427. [PubMed: 11986988]
- Kalsi G, McQuillin A, Degen B, Lundorf MD, Bass NJ, Lawrence J, Choudhury K, Puri V, Nyegaard M, Curtis D, Mors O, Kruse T, Kerwin S, Gurling H. Identification of the Slynar gene (AY070435) and related brain expressed sequences as a candidate gene for susceptibility to affective disorders through allelic and haplotypic association with bipolar disorder on chromosome 12q24. *The American journal of psychiatry*. 2006; 163(10):1767–1776. [PubMed: 17012688]
- Kapur S. Psychosis as a state of aberrant salience: a framework linking biology, phenomenology, and pharmacology in schizophrenia. *The American journal of psychiatry*. 2003; 160(1):13–23. [PubMed: 12505794]
- Kasperek T, Marecek R, Schwarz D, Prikryl R, Vanicek J, Mikl M, Ceskova E. Source-based morphometry of gray matter volume in men with first-episode schizophrenia. *Human brain mapping*. 2010; 31(2):300–310. [PubMed: 19777553]
- Kochunov P, Davis M. Development of structural MR brain imaging protocols to study genetics and maturation. *Methods*. 2009; 50(3):136–146. [PubMed: 19665566]
- Kochunov P, Lancaster JL, Glahn DC, Purdy D, Laird AR, Gao F, Fox P. Retrospective motion correction protocol for high-resolution anatomical MRI. *Hum Brain Mapp*. 2006; 27(12):957–962. [PubMed: 16628607]

- Li YO, Adali T, Calhoun VD. Estimating the number of independent components for functional magnetic resonance imaging data. *Human brain mapping*. 2007; 28(11):1251–1266. [PubMed: 17274023]
- Lien YJ, Liu CM, Faraone SV, Tsuang MT, Hwu HG, Hsiao PC, Chen WJ. A genome-wide quantitative trait loci scan of neurocognitive performances in families with schizophrenia. *Genes, brain, and behavior*. 2010; 9(7):695–702.
- Lyall AE, Shi F, Geng X, Woolson S, Li G, Wang L, Hamer RM, Shen D, Gilmore JH. Dynamic Development of Regional Cortical Thickness and Surface Area in Early Childhood. *Cerebral cortex*. 2014
- McInnis MG, Lan TH, Willour VL, McMahon FJ, Simpson SG, Addington AM, MacKinnon DF, Potash JB, Mahoney AT, Chellis J, Huo Y, Swift-Scanlan T, Chen H, Koskela R, Stine OC, Jamison KR, Holmans P, Folstein SE, Ranade K, Friddle C, Botstein D, Marr T, Beaty TH, Zandi P, DePaulo JR. Genome-wide scan of bipolar disorder in 65 pedigrees: supportive evidence for linkage at 8q24, 18q22, 4q32, 2p12, and 13q12. *Molecular psychiatry*. 2003; 8(3):288–298. [PubMed: 12660801]
- McKay DR, Knowles EE, Winkler AA, Sprooten E, Kochunov P, Olvera RL, Curran JE, Kent JW Jr. Carless MA, Goring HH, Dyer TD, Duggirala R, Almasy L, Fox PT, Blangero J, Glahn DC. Influence of age, sex and genetic factors on the human brain. *Brain imaging and behavior*. 2014; 8(2):143–152. [PubMed: 24297733]
- Mitchell BD, Kammerer CM, Blangero J, Mahaney MC, Rainwater DL, Dyke B, Hixson JE, Henkel RD, Sharp RM, Comuzzie AG, VandeBerg JL, Stern MP, MacCluer JW. Genetic and environmental contributions to cardiovascular risk factors in Mexican Americans. The San Antonio Family Heart Study. *Circulation*. 1996; 94(9):2159–2170. [PubMed: 8901667]
- Moises HW, Yang L, Kristbjarnarson H, Wiese C, Byerley W, Macciardi F, Arolt V, Blackwood D, Liu X, Sjogren B, et al. An international two-stage genome-wide search for schizophrenia susceptibility genes. *Nature genetics*. 1995; 11(3):321–324. [PubMed: 7581457]
- Morissette J, Villeneuve A, Bordeleau L, Rochette D, Laberge C, Gagne B, Laprise C, Bouchard G, Plante M, Gobeil L, Shink E, Weissenbach J, Barden N. Genome-wide search for linkage of bipolar affective disorders in a very large pedigree derived from a homogeneous population in quebec points to a locus of major effect on chromosome 12q23-q24. *American journal of medical genetics*. 1999; 88(5):567–587. [PubMed: 10490718]
- Moskvina V, Schmidt KM. On multiple-testing correction in genome-wide association studies. *Genetic epidemiology*. 2008; 32(6):567–573. [PubMed: 18425821]
- Olvera RL, Bearden CE, Velligan DI, Almasy L, Carless MA, Curran JE, Williamson DE, Duggirala R, Blangero J, Glahn DC. Common genetic influences on depression, alcohol, and substance use disorders in Mexican-American families. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*. 2011; 156B(5):561–568.
- Palaniyappan L, Balain V, Liddle PF. The neuroanatomy of psychotic diathesis: a meta-analytic review. *Journal of psychiatric research*. 2012; 46(10):1249–1256. [PubMed: 22790253]
- Pu W, Li L, Zhang H, Ouyang X, Liu H, Zhao J, Li L, Xue Z, Xu K, Tang H, Shan B, Liu Z, Wang F. Morphological and functional abnormalities of salience network in the early-stage of paranoid schizophrenia. *Schizophrenia research*. 2012; 141(1):15–21. [PubMed: 22910405]
- Rimol LM, Nesvag R, Hagler DJ Jr, Bergmann O, Fennema-Notestine C, Hartberg CB, Haukvik UK, Lange E, Pung CJ, Server A, Melle I, Andreassen OA, Agartz I, Dale AM. Cortical volume, surface area, and thickness in schizophrenia and bipolar disorder. *Biological psychiatry*. 2012; 71(6):552–560. [PubMed: 22281121]
- Rose EJ, Greene C, Kelly S, Morris DW, Robertson IH, Fahey C, Jacobson S, O'Doherty J, Newell FN, McGrath J, Bokde A, Garavan H, Frodl T, Gill M, Corvin AP, Donohoe G. The NOS1 variant rs6490121 is associated with variation in prefrontal function and grey matter density in healthy individuals. *NeuroImage*. 2012; 60(1):614–622. [PubMed: 22227051]
- Schizophrenia Working Group of the Psychiatric Genomics C. Biological insights from 108 schizophrenia-associated genetic loci. *Nature*. 2014; 511(7510):421–427. [PubMed: 25056061]
- Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, Reiss AL, Greicius MD. Dissociable intrinsic connectivity networks for salience processing and executive control. *The*

Journal of neuroscience : the official journal of the Society for Neuroscience. 2007; 27(9):2349–2356. [PubMed: 17329432]

Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar GC. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. The Journal of clinical psychiatry. 1998; 59(Suppl 20):22–33. quiz 34–57. [PubMed: 9881538]

Shepherd AM, Laurens KR, Matheson SL, Carr VJ, Green MJ. Systematic meta-review and quality assessment of the structural brain alterations in schizophrenia. Neuroscience and biobehavioral reviews. 2012; 36(4):1342–1356. [PubMed: 22244985]

Shink E, Harvey M, Tremblay M, Gagne B, Belleau P, Raymond C, Labbe M, Dube MP, Lafreniere RG, Barden N. Analysis of microsatellite markers and single nucleotide polymorphisms in candidate genes for susceptibility to bipolar affective disorder in the chromosome 12Q24.31 region. American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics. 2005; 135B(1):50–58.

Silberberg G, Ben-Shachar D, Navon R. Genetic analysis of nitric oxide synthase 1 variants in schizophrenia and bipolar disorder. American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics. 2010; 153B(7):1318–1328.

Sowell ER, Thompson PM, Leonard CM, Welcome SE, Kan E, Toga AW. Longitudinal mapping of cortical thickness and brain growth in normal children. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2004; 24(38):8223–8231. [PubMed: 15385605]

Sprooten E, Knowles EE, McKay DR, Goring HH, Curran JE, Kent JW Jr. Carless MA, Dyer TD, Drigalenko EI, Olvera RL, Fox PT, Almasy L, Duggirala R, Kochunov P, Blangero J, Glahn DC. Common genetic variants and gene expression associated with white matter microstructure in the human brain. NeuroImage. 2014; 97:252–261. [PubMed: 24736177]

Stein JL, Medland SE, Vasquez AA, Hibar DP, Senstad RE, Winkler AM, Toro R, Appel K, Barteczek R, Bergmann O, Bernard M, Brown AA, Cannon DM, Chakravarty MM, Christoforou A, Domin M, Grimm O, Hollinshead M, Holmes AJ, Homuth G, Hottenga JJ, Langan C, Lopez LM, Hansell NK, Hwang KS, Kim S, Laje G, Lee PH, Liu X, Loth E, Lourdasamy A, Mattingdal M, Mohnke S, Maniega SM, Nho K, Nugent AC, O'Brien C, Papmeyer M, Putz B, Ramasamy A, Rasmussen J, Rijpkema M, Risacher SL, Roddey JC, Rose EJ, Ryten M, Shen L, Sprooten E, Strengman E, Teumer A, Trabzuni D, Turner J, van Eijk K, van Erp TG, van Tol MJ, Wittfeld K, Wolf C, Woudstra S, Aleman A, Alhusaini S, Almasy L, Binder EB, Brohawn DG, Cantor RM, Carless MA, Corvin A, Cizisch M, Curran JE, Davies G, de Almeida MA, Delanty N, Depondt C, Duggirala R, Dyer TD, Erk S, Fagerness J, Fox PT, Freimer NB, Gill M, Goring HH, Hagler DJ, Hoehn D, Holsboer F, Hoogman M, Hosten N, Jahanshad N, Johnson MP, Kasperaviciute D, Kent JW Jr. Kochunov P, Lancaster JL, Lawrie SM, Liewald DC, Mandl R, Matarin M, Mattheisen M, Meisenzahl E, Melle I, Moses EK, Muhleisen TW, Nauck M, Nothen MM, Olvera RL, Pandolfo M, Pike GB, Puls R, Reinvang I, Renteria ME, Rietschel M, Roffman JL, Royle NA, Rujescu D, Savitz J, Schnack HG, Schnell K, Seiferth N, Smith C, Steen VM, Valdes Hernandez MC, Van den Heuvel M, van der Wee NJ, Van Haren NE, Veltman JA, Volzke H, Walker R, Westlye LT, Whelan CD, Agartz I, Boomsma DI, Cavalleri GL, Dale AM, Djurovic S, Drevets WC, Hagoort P, Hall J, Heinz A, Jack CR Jr. Foroud TM, Le Hellard S, Macciardi F, Montgomery GW, Poline JB, Porteous DJ, Sisodiya SM, Starr JM, Sussmann J, Toga AW, Veltman DJ, Walter H, Weiner MW, Alzheimer's Disease Neuroimaging I; Consortium E; Consortium I; Saguenay Youth Study G; Bis JC, Ikram MA, Smith AV, Gudnason V, Tzourio C, Vernooij MW, Launer LJ, DeCarli C, Seshadri S, Cohorts for H; Aging Research in Genomic Epidemiology C; Andreassen OA, Apostolova LG, Bastin ME, Blangero J, Brunner HG, Buckner RL, Cichon S, Coppola G, de Zubicaray GI, Deary IJ, Donohoe G, de Geus EJ, Espeseth T, Fernandez G, Glahn DC, Grabe HJ, Hardy J, Hulshoff Pol HE, Jenkinson M, Kahn RS, McDonald C, McIntosh AM, McMahon FJ, McMahon KL, Meyer-Lindenberg A, Morris DW, Muller-Myhsok B, Nichols TE, Ophoff RA, Paus T, Pausova Z, Penninx BW, Potkin SG, Samann PG, Saykin AJ, Schumann G, Smoller JW, Wardlaw JM, Weale ME, Martin NG, Franke B, Wright MJ, Thompson PM, Enhancing Neuro Imaging Genetics through Meta-Analysis C. Identification of common variants associated with human hippocampal and intracranial volumes. Nature genetics. 2012; 44(5):552–561. [PubMed: 22504417]

- Stephan KE, Friston KJ, Frith CD. Dysconnection in schizophrenia: from abnormal synaptic plasticity to failures of self-monitoring. *Schizophrenia bulletin*. 2009; 35(3):509–527. [PubMed: 19155345]
- Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Archives of general psychiatry*. 2003; 60(12):1187–1192. [PubMed: 14662550]
- Turner JA, Calhoun VD, Michael A, van Erp TG, Ehrlich S, Segall JM, Gollub RL, Csernansky J, Potkin SG, Ho BC, Bustillo J, Schulz SC, Fbirt, Wang L. Heritability of multivariate gray matter measures in schizophrenia. *Twin research and human genetics : the official journal of the International Society for Twin Studies*. 2012; 15(3):324–335. [PubMed: 22856368]
- Verrall L, Burnet PW, Betts JF, Harrison PJ. The neurobiology of D-amino acid oxidase and its involvement in schizophrenia. *Molecular psychiatry*. 2010; 15(2):122–137. [PubMed: 19786963]
- Williams JT, Blangero J. Comparison of variance components and sibpair-based approaches to quantitative trait linkage analysis in unselected samples. *Genetic epidemiology*. 1999; 16(2):113–134. [PubMed: 10030396]
- Williams NM, Norton N, Williams H, Ekholm B, Hamshere ML, Lindblom Y, Chowdari KV, Cardno AG, Zammit S, Jones LA, Murphy KC, Sanders RD, McCarthy G, Gray MY, Jones G, Holmans P, Nimgaonkar V, Adolfson R, Osby U, Terenius L, Sedvall G, O'Donovan MC, Owen MJ. A systematic genomewide linkage study in 353 sib pairs with schizophrenia. *American journal of human genetics*. 2003; 73(6):1355–1367. [PubMed: 14628288]
- Winkler AM, Kochunov P, Blangero J, Almasy L, Zilles K, Fox PT, Duggirala R, Glahn DC. Cortical thickness or grey matter volume? The importance of selecting the phenotype for imaging genetics studies. *NeuroImage*. 2010; 53(3):1135–1146. [PubMed: 20006715]
- Wockner LF, Noble EP, Lawford BR, Young RM, Morris CP, Whitehall VL, Voisey J. Genome-wide DNA methylation analysis of human brain tissue from schizophrenia patients. *Translational psychiatry*. 2014; 4:e339. [PubMed: 24399042]
- Xu L, Groth KM, Pearlson G, Schretlen DJ, Calhoun VD. Source-based morphometry: the use of independent component analysis to identify gray matter differences with application to schizophrenia. *Human brain mapping*. 2009; 30(3):711–724. [PubMed: 18266214]

Figure 1. The insula-mPFC component derived from SBM analysis in GOBS

Insula-mPFC component map showing the voxels that primarily contribute to the component of interest, and that covary highly with one another across individuals in the GOBS sample. Voxels loading positively ($z > 2.5$) on the component are colored red-to-yellow, and voxels loading negatively ($z < -2.5$) are colored blue-to-light blue. Images are in radiological convention.

Figure 2. Linkage peak on chromosome 12 at 12q24 for weights on the insula - medial prefrontal cortex component

LOD scores plotted against location on chromosome 12. The maximum LOD is 3.76 at 12q24. The threshold for genome-wide significance is at 2.9, as indicated by the dashed line.