Psychological Medicine (2016), **46**, 1523–1534. © Cambridge University Press 2016
doi:10.1017/S0033291716000167

ORIGINAL ARTICLE

The *CHRM3* gene is implicated in abnormal thalamo-orbital frontal cortex functional connectivity in first-episode treatment-naive patients with schizophrenia

Q. Wang[1,2]†, W. Cheng[3]†, M. Li[4]†, H. Ren[1], X. Hu[5], W. Deng[1], M. Li[1], X. Ma[2], L. Zhao[2], Y. Wang[2], B. Xiang[1], H.-M. Wu[4], P. C. Sham[4], J. Feng[3]* and T. Li[1,2]*

[1] *Mental Health Center, West China Hospital, Sichuan University, Chengdu, Sichuan, People's Republic of China*
[2] *State Key Laboratory of Biotherapy, Psychiatric Laboratory, West China Hospital, Sichuan University, Chengdu, Sichuan, People's Republic of China*
[3] *Centre for Computational Systems Biology, Fudan University, Shanghai, People's Republic of China*
[4] *State Key Laboratory of Brain and Cognitive Sciences, Centre for Genomic Sciences and Department of Psychiatry, University of Hong Kong, Pokfulam, S.A.R. China*
[5] *Biobank, West China Hospital, Sichuan University, Chengdu, Sichuan, People's Republic of China*

Background. The genetic influences in human brain structure and function and impaired functional connectivities are the hallmarks of the schizophrenic brain. To explore how common genetic variants affect the connectivities in schizophrenia, we applied genome-wide association studies assaying the abnormal neural connectivities in schizophrenia as quantitative traits.

Method. We recruited 161 first-onset and treatment-naive patients with schizophrenia and 150 healthy controls. All the participants underwent scanning with a 3 T-magnetic resonance imaging scanner to acquire structural and functional imaging data and genotyping using the HumanOmniZhongHua-8 BeadChip. The brain-wide association study approach was employed to account for the inherent modular nature of brain connectivities.

Results. We found differences in four abnormal functional connectivities [left rectus to left thalamus (REC.L–THA.L), left rectus to right thalamus (REC.L–THA.R), left superior orbital cortex to left thalamus (ORBsup.L–THA.L) and left superior orbital cortex to right thalamus (ORBsup.L–THA.R)] between the two groups. Univariate single nucleotide polymorphism (SNP)-based association revealed that the SNP rs6800381, located nearest to the *CHRM3* (cholinergic receptor, muscarinic 3) gene, reached genomic significance ($p = 1.768 \times 10^{-8}$) using REC.L–THA.R as the phenotype. Multivariate gene-based association revealed that the *FAM12A* (family with sequence similarity 12, member A) gene nearly reached genomic significance (nominal $p = 2.22 \times 10^{-6}$, corrected $p = 0.05$).

Conclusions. Overall, we identified the first evidence that the *CHRM3* gene plays a role in abnormal thalamo-orbital frontal cortex functional connectivity in first-episode treatment-naive patients with schizophrenia. Identification of these genetic variants using neuroimaging genetics provides insights into the causes of variability in human brain development, and may help us determine the mechanisms of dysfunction in schizophrenia.

Received 20 November 2015; Revised 8 January 2016; Accepted 12 January 2016

Key words: First-episode patients, functional connectivity, genome-wide association studies, schizophrenia.

Introduction

Schizophrenia is one of the most common disabling mental illnesses. It has been known to affect 1% of the population worldwide, and causes heavy burden on families and society (van Os & Kapur, 2009). Although many studies, including family and twin studies, have shown that schizophrenia has high heritability (80%), schizophrenia still cannot be explained as a monogenic disorder (McGuffin *et al.* 1984; Sawa & Snyder, 2002). The Psychiatric Genome Consortium carried out a large-scale study and uncovered many genes associated with schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). It is now generally recognized that schizophrenia is a complex illness caused by multiple genetic variants and each with small to modest effect size [O'Donovan *et al.* 2008; Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011]. However, it remains unknown as

* Address for correspondence: T. Li, The Mental Health Center and the Psychiatric Laboratory, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, People's Republic of China.
(Email: xuntao26@hotmail.com)
† Authors Q.W., W.C. and M.L. contributed equally to this work.

to why all the identified variants represent only a modest proportion of the overall heritability of schizophrenia. With missing heritability yet to be revealed, some studies tried to expand their sample size to increase the power of genome-wide association studies (GWAS) (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), which, although promising, can be extremely expensive and time-consuming. Two studies in Han Chinese population suggested that some common variants are involved in the susceptibility to schizophrenia (Shi *et al.* 2011; Yue *et al.* 2011); however, no overlapping single nucleotide polymorphisms (SNPs) were detected from these two studies. One of the possible reasons for this may be that the heterogeneity of the descriptive symptoms such as the disease severity and more subtle characteristics may have been largely ignored due to the clinical diagnostic categories. Recently, Arnedo *et al.* (2015) found that 17 independent gene networks are correlated with the eight subtypes of schizophrenia. This poses a challenge to the reliability of schizophrenia as a diagnostic entity. Therefore, it is important to refine the phenotype of schizophrenia rather than its general clinical symptoms in order to unravel the complex genetic structure of schizophrenia. Some GWAS were handled to detect pathogenic genes of schizophrenia using quantitative traits (QTs) such as a composite score of six neurocognitive dimensions, blood oxygen level-dependent (BOLD) signal (Potkin *et al.* 2009*b*) and gray matter volume (Stein *et al.* 2012; Wang *et al.* 2013). The limited statistical power due to the small sample size can be increased by using a QT analysis (Potkin *et al.* 2009*b*), especially QTs that can be used to test the structural and functional deficits of the brain in schizophrenia. The highly complex structure of the human brain is strongly shaped by genetic influences (Hibar *et al.* 2015).

Schizophrenia is one of the diseases implicated in the dysconnectivity of the brain (Pettersson-Yeo *et al.* 2011), and numerous studies have attempted to locate the hypothesized aberrant networks. Wernicke suggested that dysconnectivity between distinct functional modules involving both sensorimotor and association areas of the brain generates symptoms of psychosis (Cutting & Shepherd, 1987). Some previous studies have suggested many altered regional connectivities in patients with schizophrenia, which contributed to the general acceptance of a vaguely defined 'widespread dysconnectivity' (Broyd *et al.* 2009; Kim *et al.* 2009). However, the putative 'dysconnectivity of the brain' remains elusive as no constant patterns that can reliably explain the complex and heterogeneous symptoms of schizophrenia have emerged. To date, seed-based analysis (SBA) and independent component analysis (ICA) are the most common methods used to study functional connectivity in the brain of patients with schizophrenia. SBA with *a priori* specification of brain regions is hypothesis-based analysis, only allowing for testing candidate regions by previous observations. Although the ICA approach is a data-driven approach that is well suited for novel discoveries of aberrant connectivity, it is only partially independent of prior assumptions (Joel *et al.* 2011), as the components are assumed to arise from statistically independent sources and are often selected based on prior expectations of plausible signals, e.g. large-scale networks such as the default mode network. As a result, across studies, even similarly named components have a diverse anatomical distribution, which again precludes pooled synthesis of individual studies. Therefore, to provide both greater confidence and accuracy in identifying the specific regions and their functional connections that contribute most to schizophrenia, it is important to be able to use a voxel-based brain-wide analysis strategy.

Together, the present study aimed to identify the common genetic variants underlying the dysconnectivity of the brain in schizophrenia. We first investigated the most abnormal pattern of connectivity discovered from the whole-brain data-driven search. Subsequently, the dysconnectivities of the brain were integrated into the genetic data from GWAS analysis as QTs in order to identify novel susceptibility loci for schizophrenia. The present study of the imaging genetics opens the door to unravel the complex mechanisms of abnormalities of the brain in schizophrenia.

Method

Subjects

This study included 311 participants, including 161 patients with schizophrenia (82 men and 79 women) and 150 healthy control subjects (80 men and 70 women). Table 1 summarizes the demographic and clinical characteristics of the participants. More details such as data collection are presented in the Supplementary material. The study was approved by the Ethics Committee of the West China Hospital of Sichuan University. All the patients and controls provided written informed consents.

Imaging data acquisition and processing

Preprocessing and statistical analysis of functional images were carried out using the Statistical Parametric Mapping package (SPM8, Wellcome Department for Imaging Neuroscience, London, UK). For each individual participant's dataset, the first 10

https://doi.org/10.1017/S0033291716000167 Published online by Cambridge University Press

Table 1. *Demographic and clinical characteristics of the participants*

	Gender, *n*			Handedness, *n*						
Group	Male	Female	Age, years	Right	Left	Education, years	Positive scale	Negative scale	General scale	Duration of illness, years
Controls	80	70	25.8 (8.7)	150	0	13.1 (3.3)				
Patients	82	79	24.2 (8.1)	161	0	11.9 (3.3)	24.7 (5.8)	19.6 (7.6)	46.9 (8.8)	11.8 (25.8)
Statistic	0.18		1.69			2.98				
p	0.99		0.09			0.003				

Data are given as mean (standard deviation) unless otherwise indicated.

image volumes were discarded to allow the functional magnetic resonance imaging (fMRI) signal to reach a steady state. The initial analysis included slice time correction and motion realignment. The resulting images were then spatially normalized to the Montreal Neurological Institute (MNI) echo planar imaging (EPI) template in SPM8, resampled to $3 \times 3 \times 3$ mm^3, and subsequently smoothed with an isotropic Gaussian kernel [full width at half maximum (FWHM) 8 mm]. The details of imaging data acquisition and processing are presented in the online Supplementary material.

Imaging statistical analysis

Voxel-wise and atlas-based brain-wide association study

Here, we performed whole-brain voxel-wise and atlas-based association studies as described in our previous study (Cheng *et al.* 2015). In brief, a measure for the association (*MA*) between voxel *i* and the brain disorder was defined as $MA(i) = N_\alpha$, where N_α is the number of links between voxel *i* and every other voxel in the brain that has a *p* value of less than α [in the present study, $\alpha = 0.05/(47636 \times 47635/2)$] in *t* tests. A larger value of *MA* implies a more significant alteration in functional connectivity.

The *MA* value described above shows voxels with significantly different functional connectivities between cases and controls, but not the brain regions to which these voxels have altered connectivity. In order to investigate the abnormal connectivity pattern in the functional connectivity networks in schizophrenia, all significant voxels (after Bonferroni correction) were parcellated into four isolated regions in three clusters (see online Supplementary Table S1) using an anatomical labeling (AAL) atlas. The time series were then extracted in each cluster by averaging the BOLD signals of all significant voxels within that region. The functional connectivity was evaluated between each pair of clusters using Pearson's correlation coefficient. Then, cluster-wise functional connectivity analysis on the significant voxels within each cluster was performed to compare patient groups with their respective healthy controls. We finally obtained a 4×4 symmetric matrix that shows the overall pattern of the altered connectivity patterns between these voxel clusters in the schizophrenia group.

Correlations between symptom severity and abnormal functional connectivity

We investigated whether differences in functional connectivity correlated with symptom severity as assessed by the Positive and Negative Syndrome Scale (PANSS) using Pearson's correlation, using age, gender and disease duration as covariates.

Quality control and statistics for genetic data

Genotyping and quality controls

The pipeline of genotyping and quality controls was presented in a previous study (Wang *et al.* 2013). The details are presented in the online Supplementary material.

Genotype imputation

Genotypes were phased with shapeIT to generate haplotypes (Delaneau *et al.* 2012), which were used to impute missing data using IMPUTE2 (Howie *et al.* 2009), referenced to the 1000 Genomes Project phase I dataset. Imputed missing data underwent the same quality-control steps as stated above.

Association analysis using functional connectivities as QTs

SNP-based and gene-based analysis for single QTs

A mixed linear regression with SNP* group was used in this study, $y = \beta_0 + \beta_{\text{cov}} * X_{\text{cov}} + \beta_1 * \text{group} + \beta_2\, SNP + \beta_3 * SNP * \text{group} + z_{\text{ploygene}} + e$, where y stands for

https://doi.org/10.1017/S0033291716000167 Published online by Cambridge University Press

abnormal functional connectivities; X_{cov} denotes the covariates age and gender, and $z_{polygene}$ is a random effect using the kinship matrix in addition to the usual fixed effects. Compared with the fixed-effects model, the random-effect model assists in controlling for latent heterogeneity when this heterogeneity is constant over time and correlated with independent variables. Moreover, linear mixed-effects models are often preferred over more traditional approaches such as analysis of variance (ANOVA) because of the advantage of these models in dealing with missing values. On the other hand, as a large number of variables were analysed and the complexity of the model was applied in the initial analyses of the interaction term model, we only focused on the SNPs and genes that reached genome-wide significance. Using this model, we performed the two degrees of freedom joint test for the SNP main effect and the SNP × group interaction. Although PLINK has been the most popular tool in GWAS, mixed linear modeling has not been implemented in this package yet. In this study, the MixABEL package (Aulchenko *et al.* 2007) was used to explore the mixed linear model with SNP × group interaction. This package has been used in some previous GWAS studies (Svishcheva *et al.* 2012; Basson *et al.* 2014; Fabregat-Traver *et al.* 2014; Pirastu *et al.* 2015). In addition, the genome inflation factor λ was denoted as the ratio of the observed to the expected median χ^2 (0.465). Gene-based univariate association tests using the extended Simes procedure was performed for each QT by using the program GATES (Andersson *et al.* 2015). GATES is freely available in KGG v3.0 (http://statgenpro.psychiatry.hku.hk/limx/kgg/download.php).

SNP-based and gene-based analysis for multiple QTs

GWAS are generally performed one phenotype at a time, although clinical overlaps and statistical correlations between many phenotypes occur. Multivariate analysis, in which multiple phenotypes are usually reduced to a single composite score, often results in loss of statistical power. A trait-based association test has been recommended that uses an extended Simes procedure (TATES) to overcome loss of statistical power (Li *et al.* 2011, van der Sluis *et al.* 2013). TATES combines p values obtained in univariate GWAS to generate one multi-phenotype-based p value, while correlations between components are corrected. Unlike other multivariate methods (O'Reilly *et al.* 2012), TATES unravels both genetic variants that are common to multiple phenotypes as well as phenotype-specific variants (van der Sluis *et al.* 2013). Extensive simulations show that TATES can warrant correct false-positive rates and is more powerful than the univariate tests of composite scores and the standard multivariate ANOVA (van der Sluis *et al.* 2013).

In this study, we used multivariate gene-based association by the extended Simes procedure (MGAS). This approach allows gene-based testing of multivariate phenotypes in unrelated individuals (Van der Sluis *et al.* 2015). MGAS is freely available in KGG v3.0.

Results

Whole-brain voxel-based functional networks

Fig. 1 illustrates the anatomical locations that show significant whole-brain connectivity aberrations in patients with schizophrenia and in the control subjects ($p < 4.5 \times 10^{-11}$ after Bonferroni correction). Voxels with significantly altered functional connectivities in the schizophrenia population are shown in color, assessed by the *MA* given by the number of significantly affected links relating to each voxel (Fig. 1*A*). The most significantly altered cluster was in the superior frontal gyrus (peak MNI coordinates −12, 48, −18; cluster size 45, *MA* 92), while the second most significantly altered cluster was in the thalamus (peak MNI coordinates −12, −12, 6; cluster size 69, *MA* 33). Based on the AAL template, the cluster with peak MNI coordinates (−12, 48, −18) can be parcellated into two brain regions, namely, the left rectus and the superior orbital cortex. Online Supplementary Table S1 summarizes the coordinates of the significant clusters. The bilateral thalamus, left rectus (REC.L), and left superior frontal gyrus, and the orbital frontal cortex (OFC) showed significantly abnormal functional connections in the patients with schizophrenia (Fig. 1*B*).

Altered functional connectivity pattern

Fig. 2 provides a schematic brain-based diagram of altered functional connectivities based on the significant voxels in the above-mentioned four brain regions: the left thalamus (THA.L), right thalamus (THA.R), left superior orbital cortex (ORBsup.L) and REC.L. Four significant increased functional connectivities between these brain regions were found in the schizophrenic patients ($p < 1 \times 10^{-11}$).

Correlation between clinical symptoms and altered functional connectivity

We calculated Pearson's correlation between the strength of significantly increased functional connectivity and symptom severity (positive, negative, general pathopsychological symptoms, and total PANSS scores assessed using the PANSS). As shown in online

https://doi.org/10.1017/S0033291716000167 Published online by Cambridge University Press

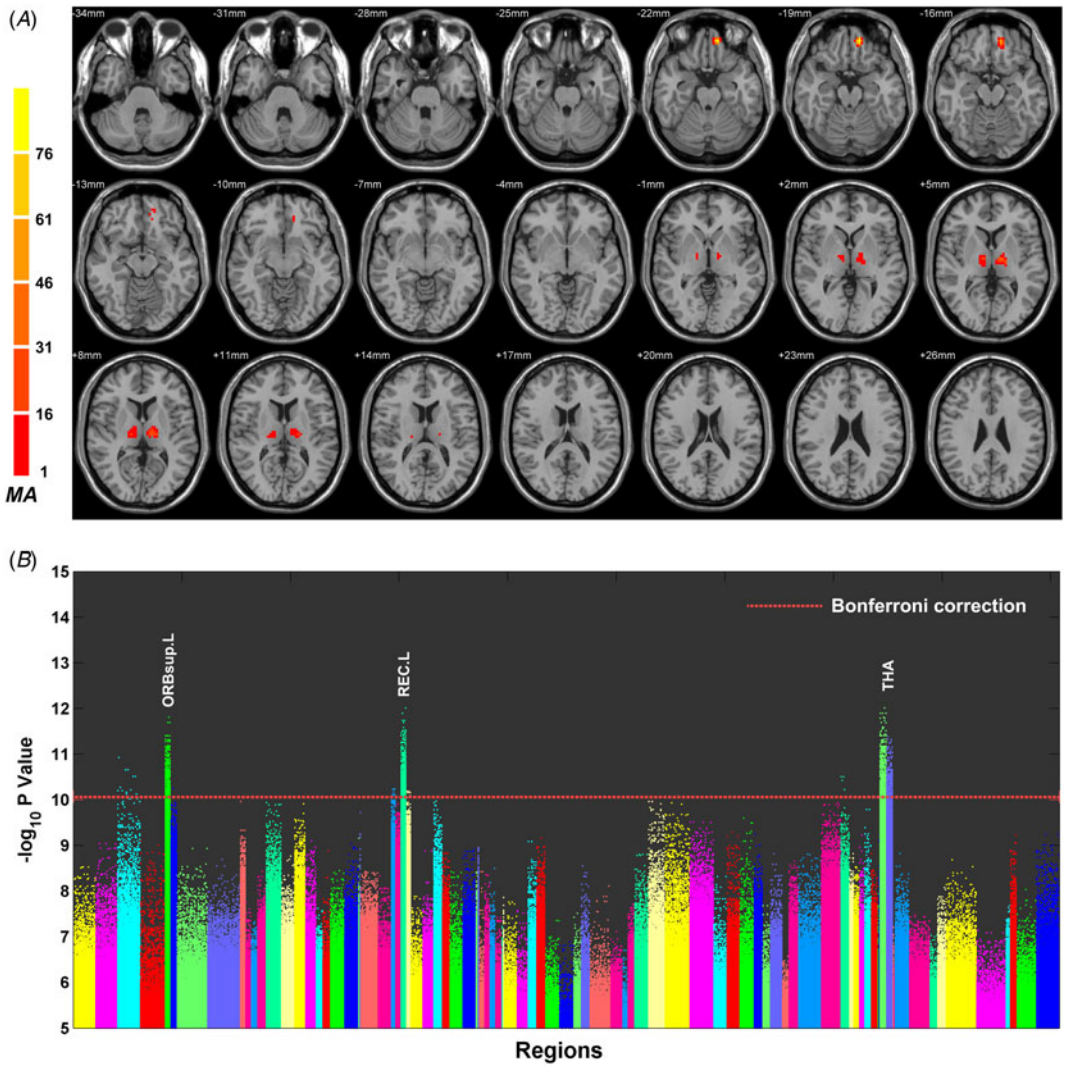

Fig. 1. Voxels with different functional connectivity in schizophrenia. (*A*) Location of the voxels with significantly altered functional connectivity with other voxels (using whole-brain Bonferroni correction). (*B*) Manhattan plot showing the probability values for each link differing between the schizophrenia and control groups. Each dot is a functional connectivity link between two voxels. There are a total of $47636 \times 47636/2$ links, and a dot is only plotted if $p < 10^{-5}$. The red dotted line is the Bonferroni correction threshold 4.4×10^{-11}. The regions indicate the anatomical labeling (AAL) areas in which the voxels were located, with the numbers for each region specified in Table 1. THA.R, Right thalamus; THR.L, left thalamus; REC.L, left rectal gyrus; ORBsup.L, left superior orbital frontal gyrus.

Supplementary Fig. S1, there was a trend of correlation between negative symptoms and the increased functional connectivity of REC.L and THA.L ($r = 0.186$, $p < 0.021$), but the correction did not survive after multiple corrections. We did not find any correlations between any of the other functional connectivities and clinical symptoms.

Results from GWAS

In all, five patients and four controls were excluded from this study after failing the cryptic relatedness test and minimal missing genotyped rates (>5%). After SNP imputation and stringent SNP quality controls, 227 subjects with high-quality genotypes (6 055 918 SNPs with an average call rate of 99.9%) remained in this study. The principal components analysis (PCA) identified no obviously different structures between cases and controls (online Supplementary Fig. S2). The QQ plot of the linear mixed-model (LMM) showed that the genomic inflation factors for the four functional connectivities after PCA adjustment (λ) were 1.0162, 1.0173, 1.099 and 1.0201, respectively (online Supplementary Fig. S3*A*–*D*), suggesting good quality controls and absence of population stratification for our samples.

https://doi.org/10.1017/S0033291716000167 Published online by Cambridge University Press

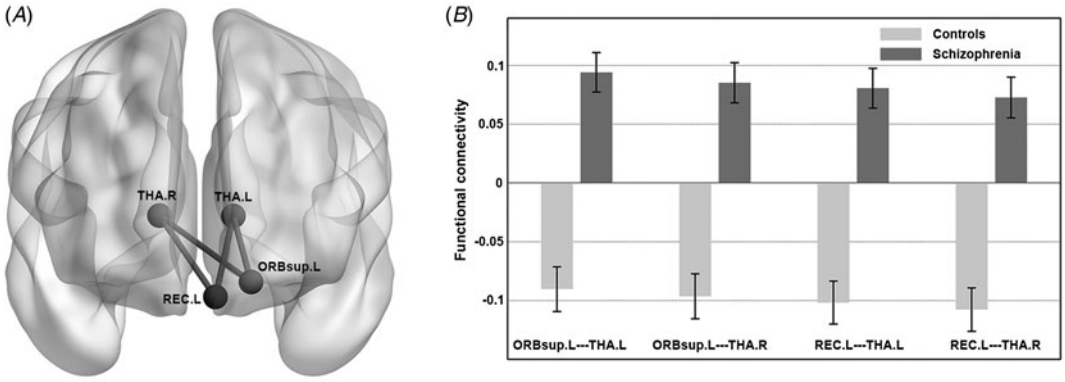

Fig. 2. Functional connectivity matrix calculated from the blood oxygen level-dependent (BOLD) signals in the significant voxels in each of the four clusters. (*A*) Schematic diagram showing differences in voxel cluster-based connectivities between the schizophrenia and control groups. (*B*) Bar plot of the four functional connectivities. Values are means, with standard deviations represented by vertical bars. THA.R, Right thalamus; THR.L, left thalamus; REC.L, left rectal gyrus; ORBsup.L, left superior orbital frontal gyrus.

SNP-based results

As shown in online Supplementary Table S2, we used an LMM statistic and complementary logistic regression analysis for an additive genetic model to estimate the effect sizes of individual SNPs. The LMM with genotype × group interaction was performed using the four increased functional connectivities (ORBsup.L–THA.L, ORBsup.L–THA.R, REC.L–THA.L, and REC.L–THA.R) as QTs. Fig. 3 shows the Manhattan plot of the genome-wide p values obtained for the LMM analysis (p_{LMM}). The SNP rs6700381, which is the approximately 1.37 Mbp downstream of the *CHRM3* (cholinergic receptor, muscarinic 3) gene, reached genomic significance ($p = 1.76802 \times 10^{-8}$ with REC.L–THA.R as the QT, and $p = 2.7211 \times 10^{-8}$ with REC.L–THA.L as the QT). Moreover, two imputed SNPs, namely, rs10158639 and rs1092587, which mapped to *CHRM3*, were implicated to be marginally associated with the increased functional connectivities of the four QTs (p values ranged from 3.279×10^{-6} to 1.880×10^{-7}; details are shown in online Supplementary Table S2).

In the present study, we found the joint effect of multi-phenotype (i.e. increased functional connectivities of ORBsup.L–THA.L, ORBsup.L–THA.R, REC.L–THA.L, and REC.L–THA.R) using TATES. The p value of the SNP rs6700381 almost reached genome-wide significance (5.13×10^{-8}), and the p values of the SNPs rs970014 and rs970015 on gene *FAM12A* (family with sequence similarity 12, member A) reached 4.14×10^{-7} (see online Supplementary Table S2).

Gene-based results

Univariate gene-based analysis revealed that the *FAM12A* gene was significantly associated with increased functional connectivities of REC.L–THA.L and REC.L–THA.R as QTs, respectively (nominal p value $= 1.54 \times 10^{-6}$, corrected $p = 0.036$ for REC.L–THA.L; nominal $p = 7.59 \times 10^{-7}$, corrected $p = 0.018$ for REC.L–THA.R).

In multivariate gene-based analysis for the joint effect of the aforementioned four increased functional connectivities, the *FAM12A* gene also showed a significant association (nominal $p = 1.47 \times 10^{-6}$, corrected $p = 0.034$).

Discussion

In this study, we identified the altered functional network involving the bilateral thalamus and OFC in first-episode treatment-naive patients with schizophrenia. Moreover, these abnormally increased functional connectivities were independent of clinical manifestations, except negative symptoms, suggesting that the increased connectivities may be one of the endophenotypes of schizophrenia. In addition, we detected a number of genes implicated in the pathogenesis of schizophrenia using these abnormal functional connectivities as QTs, among which *CHRM3* was the most associated gene, reaching genomic significance in univariate analysis and almost reaching genomic significance in multivariate analysis. In the gene-based analysis, the *FAM12A* gene reaches a genomic significance in univariate and multivariate analysis.

The thalamus and OFC were highlighted as the most altered regions in functional brain networks in the present study. Consistent with previous studies, our findings support that the OFC plays an important role in the pathogenesis of schizophrenia (Nakamura *et al.* 2008) as well as the distributed functional dysconnectivity involving some brain region of the frontal

https://doi.org/10.1017/S0033291716000167 Published online by Cambridge University Press

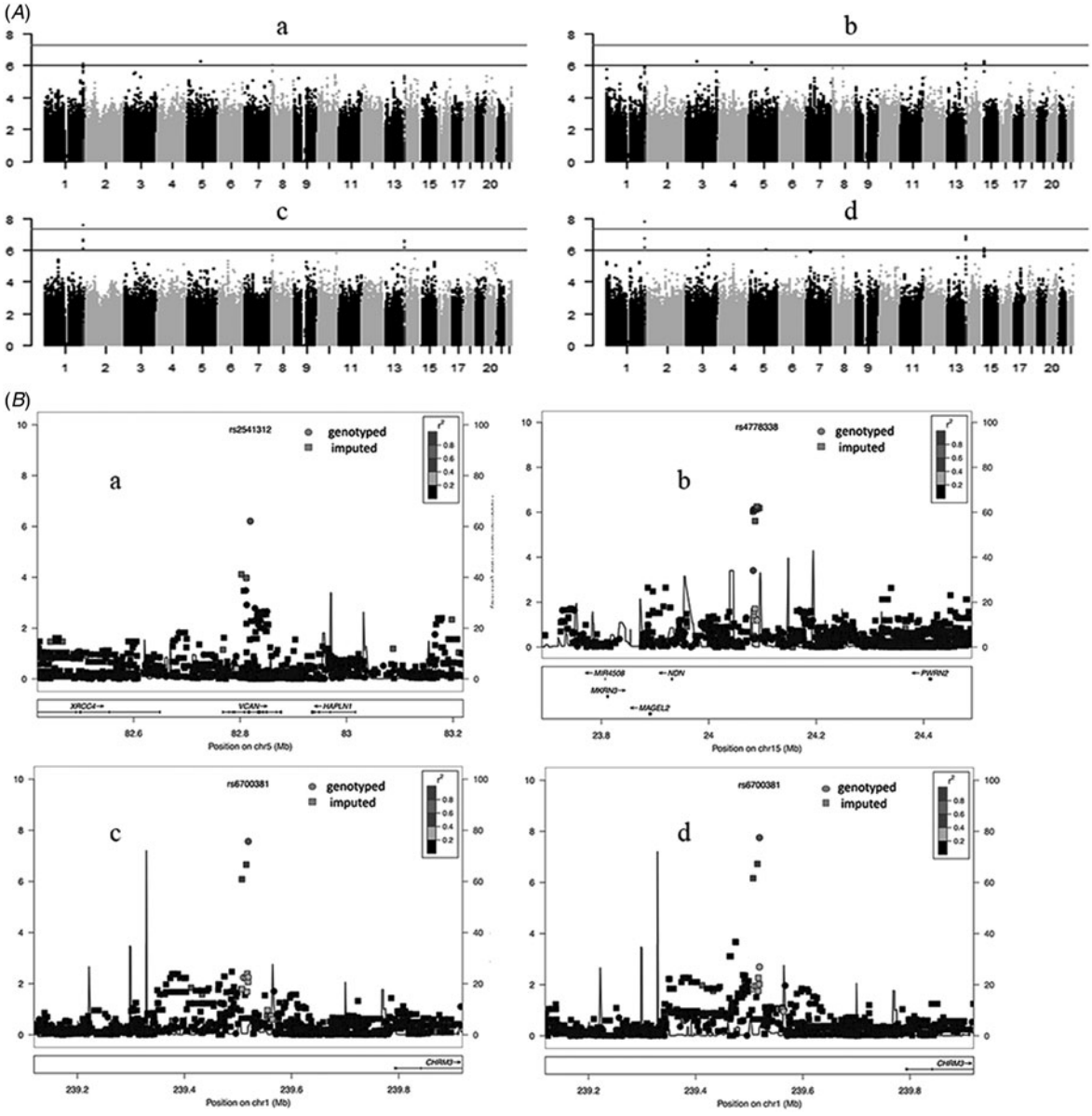

Fig. 3. (*A*) Manhattan plots for genome-wide association studies (GWAS) in 161 patients with schizophrenia and 150 controls. $\text{Log}_{10}\, p_{\text{LMM}}$ values of single nucleotide polymorphisms (SNPs) were obtained by a linear mixed-model (LMM) analysis and plotted chromosome-wise against the physical position of each SNP using four increased functional connectivities as quantitative traits (QTs). (*B*) Significance of GWAS genotyped and imputed SNPs within the 400-kb region in the *CHRM3* (cholinergic receptor, muscarinic 3) gene. All plots were adapted from LocusZoom output (Pruim *et al.* 2010).

lobe (Weinberger *et al.* 2001; Potkin *et al.* 2009*a*). The OFC is a prefrontal cortex (PFC) region in the frontal lobes and is involved in sensory integration, representing the affective value of reinforcers, and in the cognitive process of decision-making (Morten, 2005). Moreover, the thalamus plays a central and dynamic role in information transmission and processing in the brain. A series of evidence showed that the thalamus plays a vital role in the pathogenesis of schizophrenia (Andreasen *et al.* 1996; Chun *et al.* 2014). First, postmortem studies of schizophrenia patients revealed a decrease in the size of the thalamus in schizophrenia (Byne *et al.* 2002). Second, some neurocognitive performance, such as sensory gating, working memory and executive function, have been shown to activate abnormal fMRI performances of the thalamus in patients with schizophrenia, highlighting the role of the thalamus in this disorder (Andrews *et al.* 2006). Third, sleep studies have repeatedly detected a decrease in sleep spindle measures in

https://doi.org/10.1017/S0033291716000167 Published online by Cambridge University Press

patients with schizophrenia (Ferrarelli *et al.* 2010). Sleep spindles are waxing and waning 12–16 Hz oscillations initiated by the thalamic reticular nucleus and regulated by the reticulo-thalamocortical circuits (Ferrarelli *et al.* 2010).

Together, our study supports that the pathological activation of thalamus–OFC connectivity is a core feature in first-onset and drug-naive patients with schizophrenia. A growing body of evidence indicates significant abnormalities in thalamocortical connectivity in schizophrenia (Anticevic *et al.* 2014), as part of the thalamo-cortico-striatal circuits. A distinct feature of the identified thalamic connections shows a pattern of prefrontal reduction in connectivity. This pattern was first reported by Woodward *et al.* (2012) and was subsequently replicated and shown to be a common feature in bipolar disorder and schizophrenia in seed-based fMRI analyses but not data-driven analysis (Anticevic *et al.* 2014). In addition, Welsh *et al.* (2008) found significantly reduced thalamocortical connectivity in patients with chronic schizophrenia (mean duration of illness, 20.2 years) compared with matched healthy controls. However, it must be emphasized that all patients with schizophrenia recruited in these studies were chronic cases or were taking medication at the time of the study. In a longitudinal study, Anticevic *et al.* (2015) found that the PFC connectivity in early course patients with schizophrenia was increased; however, the initial hyperconnectivity is decreased with time, which may due to therapeutic effects. Antipsychotics have been found to recover the abnormally increased thalamocortical connectivities in schizophrenia (Celada *et al.* 2013). Whether or not antipsychotic use and illness duration should be included as factors that can reduce the thalamocortical connectivity remains to be an unresolved issue. Although altered functional connectivity involving the thalamus and frontal cortex in schizophrenia has been described previously (Petersen *et al.* 2013), the present investigation described many other differences, perhaps partly because in the present study we only include first-onset and treatment-naive patients, and partly due to the analysis we performed. For example, the fixed-effects models in meta-analysis consider all datasets as homogeneous and some specific altered pattern in first-onset and drug-naive group may be covered by other datasets. In addition, the frontal region is relative large and the thalamus consists of complex thalamic nuclei that have specific brain connections; therefore, the altered patterns between the thalamic nuclei and distinct frontal regions may differ across individuals. For schizophrenia, hyperconnectivity between the thalamus and frontal cortex could lead to excessive transfer of information to the frontal cortex with the compromise of losing enough thalamic control on motor/sensory information processing (Klingner *et al.* 2014).

In our study, the SNP rs6700381 in 1q43, where the *CHRM3* gene is located, was detected to associate with its role in multiple brain functional connectivities. The *CHRM3* gene decodes cholinergic receptor, muscarinic 3. The muscarinic cholinergic receptors belong to a larger family of G protein-coupled receptors. The M_3 muscarinic receptor influences a multitude of central and peripheral nervous system processes via its interaction with acetylcholine, and may be an important modulator of behavior, learning and memory. Several lines of evidence have shown that the M_3 muscarinic receptor is involved in the pathogenesis of mental illnesses. Gibbons *et al.* (2009) found that *CHRM3* gene expression is decreased in the rostral PFC of patients with bipolar disorder compared with those with depression. The rostral PFC is the region of the brain implicated in the pathogenesis of mania (Blumberg *et al.* 1999). Furthermore, there is a large overlap in the genetic backgrounds in patients with schizophrenia and bipolar disorder (Cross-Disorder Group of the Psychiatric Genomics Consortium *et al.* 2013). Therefore, it would not be surprising if the same gene is implicated in the pathogenesis of both these conditions. Also, Poulin *et al.* (2010) found that *CHRM3*-knockout mice showed a deficit in fear conditioning learning and memory, the mechanisms of which include reduction in receptor phosphorylation. It is possible that the M_3 muscarinic receptor-dependent learning and memory depend on receptor phosphorylation/arrestin signaling. Additionally, *CHRM3* has been suggested to be a candidate gene responsible for patients with 1q43 or 1q43–q44 deletions (Petersen *et al.* 2013). Moreover, deletions of 1q43 usually result in complex clinical phenotypes which include intellectual disability, autism (Petersen *et al.* 2013), seizures (EPICURE Consortium *et al.* 2012), microcephaly/craniofacial dysmorphology, corpus callosal agenesis/hypogenesis, and so on. To the best of our knowledge, our study is the first to implicate the role of *CHRM3* in schizophrenia.

The *FAM12A* gene has been previously implicated in male infertility (Damyanova *et al.* 2013). To date, this gene has not been reported to be involved in the pathogenesis of any psychiatric diseases.

Several strengths and limitations of this study must be taken into account when interpreting the findings of this study. In our study, we performed the analysis only in first-onset and treatment-naive patients with schizophrenia, which is superior to the sample populations of previous studies. We concurrently used functional connectivity assessments of both the genotype and phenotype to identify their associations, thereby combining genome and phenome information.

https://doi.org/10.1017/S0033291716000167 Published online by Cambridge University Press

Moreover, our study is data-driven rather than hypothesis-driven analysis and SBA. One limitation of this study is its sample size, which is not very large for genetic analysis. Thus, some variants could be missed due to limited statistical power. Fortunately, the endophenotype strategy may increase the power of the analysis for GWAS in the present study. The imputed genotyping data used in this study might also improve the statistical power of the analysis.

Taken together, specific alterations in resting-state thalamocortical functional connectivity are a core feature of schizophrenia. Alterations in this schizophrenia-associated network could be a reliable mechanistic index to discriminate patients from healthy controls. Furthermore, we discovered several common genetic variants underlying the abnormal increases in the functional connectivities in patients with schizophrenia. In particular, *CHRM3* could play a vital role in the deficits of thalamus–cortical connectivities. The application of neuroimaging genetics can provide insight into the causes of variability in human brain development and potentially help determine the mechanisms of schizophrenia dysfunction by unraveling susceptibility variants related to the disorder. However, our findings, of course, require a careful reconsideration of the concept of 'replicability' due to the heterogeneity and complexity of schizophrenia. In order to be meaningful in complex disorders like schizophrenia, efforts to replicate the findings of this study must take into account the distributed heritability and developmental complexity of the disease.

Supplementary material

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0033291716000167

Acknowledgements

This work was partly funded by the National Nature Science Foundation of China Key Project 81130024 (to T.L.); National Key Technology R & D Program of the Ministry of Science and Technology of China during the 12th Five-Year Plan 2012BAI01B06 (to T.L.); National Natural Science Foundation of China/Research Grants Council of Hong Kong Joint Research Scheme8141101084&N HKU736/14 (to T.L. and P.C.S.); the '135' Project of Building Top Disciplines in China of West China Hospital, Sichuan University; National Nature Science Foundation of China Grant 81271479 (to Q.W.); the outstanding young researcher grant of Sichuan University (to Q.W.) and the Hong Kong Health and Medical Research Fund 02132236 (to X.M.)

Declaration of Interest

None.

References

Andersson MA, Ek F, Olsson R (2015). Using visual lateralization to model learning and memory in zebrafish larvae. *Scientific Reports* **5**, 8667.

Andreasen NC, O'Leary DS, Cizadlo T, Arndt S, Rezai K, Ponto LL, Watkins GL, Hichwa RD (1996). Schizophrenia and cognitive dysmetria: a positron-emission tomography study of dysfunctional prefrontal–thalamic–cerebellar circuitry. *Proceedings of the National Academy of Sciences* **93**, 9985–9990.

Andrews J, Wang L, Csernansky JG, Gado MH, Barch DM (2006). Abnormalities of thalamic activation and cognition in schizophrenia. *American Journal of Psychiatry* **163**, 463–469.

Anticevic A, Cole MW, Repovs G, Murray JD, Brumbaugh MS, Winkler AM, Savic A, Krystal JH, Pearlson GD, Glahn DC (2014). Characterizing thalamo-cortical disturbances in schizophrenia and bipolar illness. *Cerebral Cortex* **24**, 3116–3130.

Anticevic A, Hu X, Xiao Y, Hu J, Li F, Bi F, Cole MW, Savic A, Yang GJ, Repovs G, Murray JD, Wang XJ, Huang X, Lui S, Krystal JH, Gong Q (2015). Early-course unmedicated schizophrenia patients exhibit elevated prefrontal connectivity associated with longitudinal change. *Journal of Neuroscience* **35**, 267–286.

Arnedo J, Svrakic DM, Del Val C, Romero-Zaliz R, Hernández-Cuervo H; Molecular Genetics of Schizophrenia Consortium, Fanous AH, Pato MT, Pato CN, de Erausquin GA, Cloninger CR, Zwir I (2015). Uncovering the hidden risk architecture of the schizophrenias: confirmation in three independent genome-wide association studies. *American Journal of Psychiatry* **172**, 139–153.

Aulchenko YS, Ripke S, Isaacs A, van Duijn CM (2007). GenABEL: an R library for genome-wide association analysis. *Bioinformatics* **23**, 1294–1296.

Basson J, Sung YJ, Schwander K, Kume R, Simino J, de las Fuentes L, Rao D (2014). Gene–education interactions identify novel blood pressure loci in the Framingham Heart Study. *American Journal of Hypertension* **27**, 431–444.

Blumberg HP, Stern E, Ricketts S, Martinez D, de Asis J, White T, Epstein J, Isenberg N, McBride PA, Kemperman I, Emmerich S, Dhawan V, Eidelberg D, Kocsis JH, Silbersweig DA (1999). Rostral and orbital prefrontal cortex dysfunction in the manic state of bipolar disorder. *American Journal of Psychiatry* **156**, 1986–1988.

Broyd SJ, Demanuele C, Debener S, Helps SK, James CJ, Sonuga-Barke EJ (2009). Default-mode brain dysfunction in mental disorders: a systematic review. *Neuroscience and Biobehavioral Reviews* **33**, 279–296.

Byne W, Buchsbaum MS, Mattiace LA, Hazlett EA, Kemether E, Elhakem SL, Purohit DP, Haroutunian V, Jones L (2002). Postmortem assessment of thalamic nuclear volumes in subjects with schizophrenia. *American Journal of Psychiatry* **159**, 59–65.

Celada P, Llado-Pelfort L, Santana N, Kargieman L, Troyano-Rodriguez E, Riga MS, Artigas F (2013). Disruption of thalamocortical activity in schizophrenia models: relevance to antipsychotic drug action. *International Journal of Neuropsychopharmacology* **16**, 2145–2163.

Cheng W, Palaniyappan L, Li M, Kendrick KM, Zhang J, Luo Q, Liu Z, Yu R, Deng W, Wang Q, Ma X, Guo W, Francis S, Liddle P, Mayer AR, Schumann G, Li T, Feng J (2015). Voxel-based, brain-wide association study of aberrant functional connectivity in schizophrenia implicates thalamocortical circuitry. *npj Schizophrenia* **1**, 15016.

Chun S, Westmoreland JJ, Bayazitov IT, Eddins D, Pani AK, Smeyne RJ, Yu J, Blundon JA, Zakharenko SS (2014). Specific disruption of thalamic inputs to the auditory cortex in schizophrenia models. *Science* **344**, 1178–1182.

Cross-Disorder Group of the Psychiatric Genomics Consortium, Lee SH, Ripke S, Neale BM, Faraone SV, Purcell SM, Perlis RH, Mowry BJ, Thapar A, Goddard ME, Witte JS, Absher D, Agartz I, Akil H, Amin F, Andreassen OA, Anjorin A, Anney R, Anttila V, Arking DE, Asherson P, Azevedo MH, Backlund L, Badner JA, Bailey AJ, Banaschewski T, Barchas JD, Barnes MR, Barrett TB, Bass N, Battaglia A, Bauer M, Bayés M, Bellivier F, Bergen SE, Berrettini W, Betancur C, Bettecken T, Biederman J, Binder EB, Black DW, Blackwood DH, Bloss CS, Boehnke M, Boomsma DI, Breen G, Breuer R, Bruggeman R, Cormican P, Buccola NG, Buitelaar JK, Bunney WE, Buxbaum JD, Byerley WF, Byrne EM, Caesar S, Cahn W, Cantor RM, Casas M, Chakravarti A, Chambert K, Choudhury K, Cichon S, Cloninger CR, Collier DA, Cook EH, Coon H, Cormand B, Corvin A, Coryell WH, Craig DW, Craig IW, Crosbie J, Cuccaro ML, Curtis D, Czamara D, Datta S, Dawson G, Day R, De Geus EJ, Degenhardt F, Djurovic S, Donohoe GJ, Doyle AE, Duan J, Dudbridge F, Duketis E, Ebstein RP, Edenberg HJ, Elia J, Ennis S, Etain B, Fanous A, Farmer AE, Ferrier IN, Flickinger M, Fombonne E, Foroud T, Frank J, Franke B, Fraser C, Freedman R, Freimer NB, Freitag CM, Friedl M, Frisén L, Gallagher L, Gejman PV, Georgieva L, Gershon ES, Geschwind DH, Giegling I, Gill M, Gordon SD, Gordon-Smith K, Green EK, Greenwood TA, Grice DE, Gross M, Grozeva D, Guan W, Gurling H, De Haan L, Haines JL, Hakonarson H, Hallmayer J, Hamilton SP, Hamshere ML, Hansen TF, Hartmann AM, Hautzinger M, Heath AC, Henders AK, Herms S, Hickie IB, Hipolito M, Hoefels S, Holmans PA, Holsboer F, Hoogendijk WJ, Hottenga JJ, Hultman CM, Hus V, Ingason A, Ising M, Jamain S, Jones EG, Jones I, Jones L, Tzeng JY, Kähler AK, Kahn RS, Kandaswamy R, Keller MC, Kennedy JL, Kenny E, Kent L, Kim Y, Kirov GK, Klauck SM, Klei L, Knowles JA, Kohli MA, Koller DL, Konte B, Korszun A, Krabbendam L, Krasucki R, Kuntsi J, Kwan P, Landén M, Långström N, Lathrop M, Lawrence J, Lawson WB, Leboyer M, Ledbetter DH, Lee PH, Lencz T, Lesch KP, Levinson DF, Lewis CM, Li J, Lichtenstein P, Lieberman JA, Lin DY, Linszen DH, Liu C, Lohoff FW, Loo SK, Lord C, Lowe JK, Lucae S, MacIntyre DJ, Madden PA, Maestrini E, Magnusson PK, Mahon PB, Maier W, Malhotra AK, Mane SM, Martin CL, Martin NG, Mattheisen M, Matthews K, Mattingsdal M, McCarroll SA, McGhee KA, McGough JJ, McGrath PJ, McGuffin P, McInnis MG, McIntosh A, McKinney R, McLean AW, McMahon FJ, McMahon WM, McQuillin A, Medeiros H, Medland SE, Meier S, Melle I, Meng F, Meyer J, Middeldorp CM, Middleton L, Milanova V, Miranda A, Monaco AP, Montgomery GW, Moran JL, Moreno-De-Luca D, Morken G, Morris DW, Morrow EM, Moskvina V, Muglia P, Mühleisen TW, Muir WJ, Müller-Myhsok B, Murtha M, Myers RM, Myin-Germeys I, Neale MC, Nelson SF, Nievergelt CM, Nikolov I, Nimgaonkar V, Nolen WA, Nöthen MM, Nurnberger JI, Nwulia EA, Nyholt DR, O'Dushlaine C, Oades RD, Olincy A, Oliveira G, Olsen L, Ophoff RA, Osby U, Owen MJ, Palotie A, Parr JR, Paterson AD, Pato CN, Pato MT, Penninx BW, Pergadia ML, Pericak-Vance MA, Pickard BS, Pimm J, Piven J, Posthuma D, Potash JB, Poustka F, Propping P, Puri V, Quested DJ, Quinn EM, Ramos-Quiroga JA, Rasmussen HB, Raychaudhuri S, Rehnström K, Reif A, Ribasés M, Rice JP, Rietschel M, Roeder K, Roeyers H, Rossin L, Rothenberger A, Rouleau G, Ruderfer D, Rujescu D, Sanders AR, Sanders SJ, Santangelo SL, Sergeant JA, Schachar R, Schalling M, Schatzberg AF, Scheftner WA, Schellenberg GD, Scherer SW, Schork NJ, Schulze TG, Schumacher J, Schwarz M, Scolnick E, Scott LJ, Shi J, Shilling PD, Shyn SI, Silverman JM, Slager SL, Smalley SL, Smit JH, Smith EN, Sonuga-Barke EJ, St Clair D, State M, Steffens M, Steinhausen HC, Strauss JS, Strohmaier J, Stroup TS, Sutcliffe JS, Szatmari P, Szelinger S, Thirumalai S, Thompson RC, Todorov AA, Tozzi F, Treutlein J, Uhr M, van den Oord EJ, Van Grootheest G, Van Os J, Vicente AM, Vieland VJ, Vincent JB, Visscher PM, Walsh CA, Wassink TH, Watson SJ, Weissman MM, Werge T, Wienker TF, Wijsman EM, Willemsen G, Williams N, Willsey AJ, Witt SH, Xu W, Young AH, Yu TW, Zammit S, Zandi PP, Zhang P, Zitman FG, Zöllner S, Devlin B, Kelsoe JR, Sklar P, Daly MJ, O'Donovan MC, Craddock N, Sullivan PF, Smoller JW, Kendler KS, Wray NR; International Inflammatory Bowel Disease Genetics Consortium (IIBDGC) (2013). Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. *Nature Genetics* **45**, 984–994.

Cutting JC, Shepherd M (1987). *The Clinical Roots of the Schizophrenia Concept: Translations of Seminal European Contributions on Schizophrenia*. Cambridge University Press: New York.

Damyanova V, Dimitrova-Dikanarova D, Hadjidekova S, Savov A, Nesheva D, Rukova B, Vatev I, Toncheva D (2013). [Genomic study in patients with idiopathic azoospermia and oligoasthenoteratozoospermia] [article in Bulgarian]. *Akusherstvo i Ginekologiia* **52**, 27–34.

Delaneau O, Marchini J, Zagury JF (2012). A linear complexity phasing method for thousands of genomes. *Nature Methods* **9**, 179–181.

EPICURE Consortium, EMINet Consortium, Steffens M, Leu C, Ruppert AK, Zara F, Striano P, Robbiano A, Capovilla G, Tinuper P, Gambardella A, Bianchi A, La Neve A, Crichiutti G, de Kovel CG, Kasteleijn-Nolst

Trenite D, de Haan GJ, Lindhout D, Gaus V, Schmitz B, Janz D, Weber YG, Becker F, Lerche H, Steinhoff BJ, Kleefuss-Lie AA, Kunz WS, Surges R, Elger CE, Muhle H, von Spiczak S, Ostertag P, Helbig I, Stephani U, Moller RS, Hjalgrim H, Dibbens LM, Bellows S, Oliver K, Mullen S, Scheffer IE, Berkovic SF, Everett KV, Gardiner MR, Marini C, Guerrini R, Lehesjoki AE, Siren A, Guipponi M, Malafosse A, Thomas P, Nabbout R, Baulac S, Leguern E, Guerrero R, Serratosa JM, Reif PS, Rosenow F, Morzinger M, Feucht M, Zimprich F, Kapser C, Schankin CJ, Suls A, Smets K, De Jonghe P, Jordanova A, Caglayan H, Yapici Z, Yalcin DA, Baykan B, Bebek N, Ozbek U, Gieger C, Wichmann HE, Balschun T, Ellinghaus D, Franke A, Meesters C, Becker T, Wienker TF, Hempelmann A, Schulz H, Ruschendorf F, Leber M, Pauck SM, Trucks H, Toliat MR, Nurnberg P, Avanzini G, Koeleman BP, Sander T (2012). Genome-wide association analysis of genetic generalized epilepsies implicates susceptibility loci at 1q43, 2p16.1, 2q22.3 and 17q21.32. *Human Molecular Genetics* **21**, 5359–5372.

Fabregat-Traver D, Sharapov SZh, Hayward C, Rudan I, Campbell H, Aulchenko Y, Bientinesi P (2014). High-performance mixed models based genome-wide association analysis with omicABEL software. *F1000Res* **3**, 200.

Ferrarelli F, Peterson MJ, Sarasso S, Riedner BA, Murphy MJ, Benca RM, Bria P, Kalin NH, Tononi G (2010). Thalamic dysfunction in schizophrenia suggested by whole-night deficits in slow and fast spindles. *American Journal of Psychiatry* **167**, 1339–1348.

Gibbons AS, Scarr E, McLean C, Sundram S, Dean B (2009). Decreased muscarinic receptor binding in the frontal cortex of bipolar disorder and major depressive disorder subjects. *Journal of Affective Disorders* **116**, 184–191.

Hibar DP, Stein JL, Renteria ME, Arias-Vasquez A, Desrivières S, Jahanshad N, Toro R, Wittfeld K, Abramovic L, Andersson M, Aribisala BS, Armstrong NJ, Bernard M, Bohlken MM, Boks MP, Bralten J, Brown AA, Mallar Chakravarty M, Chen Q, Ching CR, Cuellar-Partida G, den Braber A, Giddaluru S, Goldman AL, Grimm O, Guadalupe T, Hass J, Woldehawariat G, Holmes AJ, Hoogman M, Janowitz D, Jia T, Kim S, Klein M, Kraemer B, Lee PH, Olde Loohuis LM, Luciano M, Macare C, Mather KA, Mattheisen M, Milaneschi Y, Nho K, Papmeyer M, Ramasamy A, Risacher SL, Roiz-Santiañez R, Rose EJ, Salami A, Sämann PG, Schmaal L, Schork AJ, Shin J, Strike LT, Teumer A, van Donkelaar MM, van Eijk KR, Walters RK, Westlye LT, Whelan CD, Winkler AM, Zwiers MP, Alhusaini S, Athanasiu L, Ehrlich S, Hakobjan MM, Hartberg CB, Haukvik UK, Heister AJ, Hoehn D, Kasperaviciute D, Liewald DC, Lopez LM, Makkinje RRR, Matarin M, Naber MA, Reese McKay D, Needham M, Nugent AC, Pütz B, Royle NA, Shen L, Sprooten E, Trabzuni D, van der Marel SS, van Hulzen KJ, Walton E, Wolf C, Almasy L, Ames D, Arepalli S, Assareh AA, Bastin ME, Brodaty H, Bulayeva KB, Carless MA, Cichon S, Corvin A, Curran JE, Czisch M (2015). Common genetic variants influence human subcortical brain structures. *Nature* **520**, 224–229.

Howie BN, Donnelly P, Marchini J (2009). A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genetics* **5**, e1000529.

Joel SE, Caffo BS, van Zijl P, Pekar JJ (2011). On the relationship between seed-based and ICA-based measures of functional connectivity. *Magnetic Resonance in Medicine* **66**, 644–657.

Kim DI, Manoach DS, Mathalon DH, Turner JA, Mannell M, Brown GG, Ford JM, Gollub RL, White T, Wible C (2009). Dysregulation of working memory and default-mode networks in schizophrenia using independent component analysis, an fBIRN and MCIC study. *Human Brain Mapping* **30**, 3795–3811.

Klingner CM, Langbein K, Dietzek M, Smesny S, Witte OW, Sauer H, Nenadic I (2014). Thalamocortical connectivity during resting state in schizophrenia. *European Archives of Psychiatry and Clinical Neuroscience* **264**, 111–119.

Li MX, Gui HS, Kwan JS, Sham PC (2011). GATES: a rapid and powerful gene-based association test using extended Simes procedure. *American Journal of Human Genetics* **88**, 283–293.

McGuffin P, Farmer AE, Gottesman II, Murray RM, Reveley AM (1984). Twin concordance for operationally defined schizophrenia: confirmation of familiality and heritability. *Archives of General Psychiatry* **41**, 541–545.

Morten LK (2005). The human orbitofrontal cortex: linking reward to hedonic experience. *Nature Reviews Neuroscience* **6**, 691–702.

Nakamura M, Nestor PG, Levitt JJ, Cohen AS, Kawashima T, Shenton ME, McCarley RW (2008). Orbitofrontal volume deficit in schizophrenia and thought disorder. *Brain* **131**, 180–195.

O'Donovan MC, Craddock N, Norton N, Williams H, Peirce T, Moskvina V, Nikolov I, Hamshere M, Carroll L, Georgieva L (2008). Identification of loci associated with schizophrenia by genome-wide association and follow-up. *Nature Genetics* **40**, 1053–1055.

O'Reilly PF, Hoggart CJ, Pomyen Y, Calboli FC, Elliott P, Jarvelin MR, Coin LJ (2012). MultiPhen: joint model of multiple phenotypes can increase discovery in GWAS. *PLOS ONE* **7**, e34861.

Petersen AK, Ahmad A, Shafiq M, Brown-Kipphut B, Fong CT, Anwar Iqbal M (2013). Deletion 1q43 encompassing only CHRM3 in a patient with autistic disorder. *European Journal of Medical Genetics* **56**, 118–122.

Pettersson-Yeo W, Allen P, Benetti S, McGuire P, Mechelli A (2011). Dysconnectivity in schizophrenia: where are we now? *Neuroscience and Biobehavioral Reviews* **35**, 1110–1124.

Pirastu N, Kooyman M, Traglia M, Robino A, Willems SM, Pistis G, Amin N, Sala C, Karssen LC, van Duijn CM, Toniolo D, Gasparini P (2015). Genome-wide association analysis on five isolated populations identifies variants of the HLA-DOA gene associated with white wine liking. *European Journal of Human Genetics* **23**, 1717–1722.

Potkin S, Turner J, Brown G, McCarthy G, Greve D, Glover G, Manoach D, Belger A, Diaz M, Wible C (2009*a*). Working memory and DLPFC inefficiency in schizophrenia: the FBIRN study. *Schizophrenia Bulletin* **35**, 19–31.

Potkin SG, Turner JA, Guffanti G, Lakatos A, Fallon JH, Nguyen DD, Mathalon D, Ford J, Lauriello J, Macciardi F (2009*b*). A genome-wide association study of schizophrenia using brain activation as a quantitative phenotype. *Schizophrenia Bulletin* **35**, 96–108.

Poulin B, Butcher A, McWilliams P, Bourgognon JM, Pawlak R, Kong KC, Bottrill A, Mistry S, Wess J, Rosethorne EM, Charlton SJ, Tobin AB (2010). The M3-muscarinic receptor regulates learning and memory in a receptor phosphorylation/arrestin-dependent manner. *Proceedings of the National Academy of Science USA* **107**, 9440–9445.

Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, Boehnke M, Abecasis GR, Willer CJ (2010). LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics* **26**, 2336–2337.

Sawa A, Snyder SH (2002). Schizophrenia: diverse approaches to a complex disease. *Science* **296**, 692–695.

Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium (2011). Genome-wide association study identifies five new schizophrenia loci. *Nature Genetics* **43**, 969–976.

Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014). Biological insights from 108 schizophrenia-associated genetic loci. *Nature* **511**, 421–427.

Shi Y, Li Z, Xu Q, Wang T, Li T, Shen J, Zhang F, Chen J, Zhou G, Ji W, Li B, Xu Y, Liu D, Wang P, Yang P, Liu B, Sun W, Wan C, Qin S, He G, Steinberg S, Cichon S, Werge T, Sigurdsson E, Tosato S, Palotie A, Nothen MM, Rietschel M, Ophoff RA, Collier DA, Rujescu D, Clair DS, Stefansson H, Stefansson K, Ji J, Wang Q, Li W, Zheng L, Zhang H, Feng G, He L (2011). Common variants on 8p12 and 1q24.2 confer risk of schizophrenia. *Nature Genetics* **43**, 1224–1227.

Stein JL, Medland SE, Vasquez AA, Hibar DP, Senstad RE, Winkler AM, Toro R, Appel K, Bartecek 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, Lourdusamy A, Mattingsdal 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, Czisch 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, Bis JC, Ikram MA, Smith AV, Gudnason V, Tzourio C, Vernooij MW, Launer LJ, DeCarli C, Seshadri S, 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 (2012). Identification of common variants associated with human hippocampal and intracranial volumes. *Nature Genetics* **44**, 552–561.

Svishcheva GR, Axenovich TI, Belonogova NM, van Duijn CM, Aulchenko YS (2012). Rapid variance components-based method for whole-genome association analysis. *Nature Genetics* **44**, 1166–1170.

Van der Sluis S, Dolan CV, Li J, Song Y, Sham P, Posthuma D, Li MX (2015). MGAS: a powerful tool for multivariate gene-based genome-wide association analysis. *Bioinformatics* **31**, 1007–1015.

van der Sluis S, Posthuma D, Dolan CV (2013). TATES: efficient multivariate genotype–phenotype analysis for genome-wide association studies. *PLoS Genetics* **9**, e1003235.

van Os J, Kapur S (2009). Schizophrenia. *Lancet* **374**, 635–645.

Wang Q, Xiang B, Deng W, Wu J, Li M, Ma X, Wang Y, Jiang L, McAlonan G, Chua SE, Sham PC, Hu X, Li T (2013). Genome-wide association analysis with gray matter volume as a quantitative phenotype in first-episode treatment-naive patients with schizophrenia. *PLOS One* **8**, e75083.

Weinberger DR, Egan MF, Bertolino A, Callicott JH, Mattay VS, Lipska BK, Berman KF, Goldberg TE (2001). Prefrontal neurons and the genetics of schizophrenia. *Biological Psychiatry* **50**, 825–844.

Welsh RC, Chen AC, Taylor SF (2008). Low-frequency BOLD fluctuations demonstrate altered thalamocortical connectivity in schizophrenia. *Schizophrenia Bulletin* **36**, 713–722.

Woodward ND, Karbasforoushan H, Heckers S (2012). Thalamocortical dysconnectivity in schizophrenia. *American Journal of Psychiatry* **169**, 1092–1099.

Yue WH, Wang HF, Sun LD, Tang FL, Liu ZH, Zhang HX, Li WQ, Zhang YL, Zhang Y, Ma CC, Du B, Wang LF, Ren YQ, Yang YF, Hu XF, Wang Y, Deng W, Tan LW, Tan YL, Chen Q, Xu GM, Yang GG, Zuo XB, Yan H, Ruan YY, Lu TL, Han X, Ma XH, Cai LW, Jin C, Zhang HY, Yan J, Mi WF, Yin XY, Ma WB, Liu Q, Kang L, Sun W, Pan CY, Shuang M, Yang FD, Wang CY, Yang JL, Li KQ, Ma X, Li LJ, Yu X, Li QZ, Huang X, Lv LX, Li T, Zhao GP, Huang W, Zhang XJ, Zhang D (2011). Genome-wide association study identifies a susceptibility locus for schizophrenia in Han Chinese at 11p11.2. *Nature Genetics* **43**, 1228–1231.