Polygenic effects of schizophrenia on hippocampal grey matter volume and hippocampus–medial prefrontal cortex functional connectivity

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Background

Schizophrenia is a complex mental disorder with high heritability and polygenic inheritance. Multimodal neuroimaging studies have also indicated that abnormalities of brain structure and function are a plausible neurobiological characterisation of schizophrenia. However, the polygenic effects of schizophrenia on these imaging endophenotypes have not yet been fully elucidated.

Aims

To investigate the effects of polygenic risk for schizophrenia on the brain grey matter volume and functional connectivity, which are disrupted in schizophrenia.

Method

Genomic and neuroimaging data from a large sample of Han Chinese patients with schizophrenia ($N = 509$) and healthy controls ($N = 502$) were included in this study. We examined grey matter volume and functional connectivity via structural and functional magnetic resonance imaging, respectively. Using the data from a recent meta-analysis of a genome-wide association study that comprised a large number of Chinese people, we calculated a polygenic risk score (PGRS) for each participant.

Results

The imaging genetic analysis revealed that the individual PGRS showed a significantly negative correlation with the hippocampal grey matter volume and hippocampus–medial prefrontal cortex functional connectivity, both of which were lower in the people with schizophrenia than in the controls. We also found that the observed neuroimaging measures showed weak but similar changes in unaffected first-degree relatives of patients with schizophrenia.

Conclusions

These findings suggested that genetically influenced brain grey matter volume and functional connectivity may provide important clues for understanding the pathological mechanisms of schizophrenia and for the early diagnosis of schizophrenia.

Declaration of interest

None.

Keywords

Schizophrenia; polygenic risk score; hippocampus; grey matter volume; functional connectivity.

Studies have shown that schizophrenia involves a highly heritable form of psychosis[1,2] and that the effect of each identified common genetic variant is very small.[2] Furthermore, the effects of specific genetic variants on the clinical diagnosis are sometimes inconsistent across studies.[3] This may be because the phenotypic characteristics of schizophrenia that are modulated by polygenic factors are heterogeneous and complex.[4] To capture the polygenic nature of complex disorders, a polygenic risk score (PGRS) – which is based on the additive effects of a great number of weak disease-related genetic variants[4] – can be used. In this way, the PGRS can effectively assess the effects of these disease-related genetic variants on various phenotypes. Neuroimaging studies have provided sufficient evidence that there are substantial structural and functional brain abnormalities in people with schizophrenia. A systematic meta-analysis found reduced grey matter volume (GMV) in people with schizophrenia in many cortical and subcortical regions such as the frontal lobe, temporal lobe, anterior cingulate and hippocampus.[5] Impaired functional activities and connectivity in some brain regions have also been widely reported. For example, a study found that the functional connectivity between the hippocampus and prefrontal cortex was impaired in people with schizophrenia.[6] However, whether and how the changes in these brain measures are genetically influenced remains unclear.

Previous studies have reported that the schizophrenia PGRS is associated with many types of neuroimaging endophenotypes, such as cortical gyrification[7] and working memory-related brain activity.[8–10] However, to the best of our knowledge few studies have reported the abnormal GMV and functional connectivity that are associated with the PGRS for schizophrenia,[11] so our aim was to investigate whether and how schizophrenia polygenic risk variants influence these brain measures. All participants were of Chinese Han ancestry, so PGRSs were derived from a meta-analysis of a genome-wide association study (GWAS) comprising a large number of Chinese people.[12] We investigated the impaired GMV and functional connectivity in people with schizophrenia compared with healthy controls and further examined the effects of schizophrenia polygenic risk on these brain measures. Finally, we collected data from unaffected first-degree relatives (who are at high genetic risk for schizophrenia) to find further evidence for the identified biomarkers related to the PGRS.

Methods

Participants

A total of 1011 unrelated and extensively evaluated people of Chinese Han ancestry (509 people with schizophrenia and 502 healthy

Table 1 Demographic and clinical characteristics

	Schizophrenia group ($n = 509$)	Healthy control group ($n = 502$)	t/χ^2	P
Age, mean (s.d.)	27.78 (6.48)	27.69 (5.91)	0.233	0.816
Gender, male/female (% females)	275/234 (46.0)	255/247 (49.2)	1.058	0.304
SDMT, mean (s.d.)	40.73 (14.62)	64.65 (12.73)	−27.562	<0.001
Forward-DS, mean (s.d.)	7.67 (1.69)	8.64 (1.45)	−9.725	<0.001
Backward-DS, mean (s.d.)	4.72 (1.80)	6.27 (1.93)	−13.051	<0.001
PANSS positive score, mean (s.d.)	24.26 (4.00)	–	–	–
PANSS negative score, mean (s.d.)	20.39 (6.20)	–	–	–
PANSS general score, mean (s.d.)	39.63 (7.29)	–	–	–
PANSS total score, mean (s.d.)	84.28 (12.57)	–	–	–
CPZ-eq (mg/day)[a], mean (s.d.)	406 (200.64)	–	–	–

Values are means (s.d.) if nothing else is specified. All group comparisons were made with t-test except for gender (χ^2). SDMT, Symbol Digital Modalities Test; forward-DS, Forward Digital Span; backward-DS, Backward Digital Span; PANSS, Positive and Negative Syndrome Scale; CPZ-eq, chlorpromazine equivalents.
a. The medication dosage was converted to CPZ-eq according to previous references.

controls) with both high-quality genetic and structural resonance imaging (MRI) neuroimaging data were included in this study (Table 1). They were recruited from six hospitals (Peking University Sixth Hospital, Beijing Huilongguan Hospital, Xijing Hospital, Henan Mental Hospital, Renmin Hospital of Wuhan University and Zhumadian Psychiatric Hospital). In accordance with the DSM-IV (1994) criteria for schizophrenia, all the people with schizophrenia were diagnosed consistently by two veteran senior psychiatrists using the Structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID-I, patient edition). In the Positive and Negative Syndrome Scale (PANSS) test, the total scores of all the people with schizophrenia were higher than 60, and the scores in at least 3 positive items were higher than 4. The exclusion criteria for the patients included: other psychiatric and cognitive disorders at the time of the study or by history; severe physical diseases, such as diabetes, thyroid diseases, hypertension or cardiac diseases; a history of epilepsy; a DSM-IV diagnosis of alcohol or drug dependence; electroconvulsive therapy within the past 6 months; a suicide attempt or symptoms of severe excitement and agitation; and being pregnant or breastfeeding. Healthy controls were also carefully screened by the SCID-I, non-patient edition. Furthermore, the healthy participants were also excluded if they or their first- or second-degree relatives had ever been diagnosed with any psychiatric disorders. After receiving a complete description of the study, all the participants and/or their legal guardians provided written informed consent. The project was approved by the Medical Research Ethics Committee of Peking University Sixth Hospital (number 2010052). The Chinese revision of the Wechsler Adult Intelligence Scale test, including the Symbol Digital Modalities Test (SDMT), Forward Digital Span (forward-DS) and Backward Digital Span (backward-DS) tests (Table 1), was also used to assess the cognitive ability of both the people with schizophrenia and the healthy controls.

To further explore the clinical significance of the endophenotypes we found, we also recruited 95 unaffected first-degree relatives (39 males, mean age 34.72 ± 9.57, age range 18.17–59.75) of the participants with schizophrenia and collected genomic and imaging data. Apart from being a first-degree relative of a person diagnosed with schizophrenia, the other inclusion and exclusion criteria were the same as those of the healthy controls.

Genotype processing

We used the EZgene Blood gDNA Miniprep Kit to extract genomic DNA from the whole blood of each participant and carried out whole-genome genotyping on Illumina Human OmniZhongHua-8 BeadChips. We then performed the genotype quality control on the Linux system using PLINK version 1.0.7.[13] First, we removed the individuals whose missing genotype rates were greater than 0.05. We then used the pairwise identity by descent estimate to

identify individuals who could possibly be related. For pairs of individuals who had more similar genotypes than we would have expected by chance in a random sample, we removed the one with the greater missing rate. Next, we used single nucleotide polymorphism (SNP)-level filtering to remove SNPs with missing genotype rates greater than 0.05, a minor allele frequency less than 0.01 and a significant departure from Hardy–Weinberg equilibrium ($P < 0.001$). To avoid the confounding effects of population stratification,[8] we performed a principal component analysis on the Linux system by using EIGENSTRAT 5.0.2[14,15] on a linkage disequilibrium pruned set of autosomal SNPs. This set was obtained by carrying out linkage disequilibrium pruning with PLINK ($r^2 < 0.05$) and removing five long-range linkage disequilibrium regions with the HapMap phase 3 reference data-set.[16] After getting 10 principal components we excluded the outliers of the samples with >6 s.d. Ungenotyped SNPs were imputed on the Linux system using SHAPEIT version 2 (r790)[17] and IMPUTE2[18] with the 1000 Genomes Phase 1 reference data-set. Further analyses focused on autosomal SNPs with imputation quality scores greater than 0.8.

Computation of PGRS

The PGRS for schizophrenia was created using the 'score' utility in PLINK. The PGRS computation method was developed by Purcell and colleagues,[4] as described in full detail in Walton and colleagues.[10] The PGRS for each participant was calculated by summing the number of risk alleles weighted by the strength of the association of each SNP with schizophrenia. The strength of the association for each SNP was measured by the risk allele effect size (natural log of the odds ratio) reported by the meta-analysis of the GWAS comprising a large number of Chinese individuals.[12] The participants included in our study were obviously independent of the participants from the meta-analysis. Overall, 17 PGRSs for each participant were obtained with the following different SNP inclusion thresholds: $P_T < 0.5$, $P_T < 0.4$, $P_T < 0.3$, $P_T < 0.2$, $P_T < 0.1$, $P_T < 0.05$, $P_T < 0.01$, $P_T < 0.005$, $P_T < 0.001$, $P_T < 5 \times 10^{-4}$, $P_T < 1 \times 10^{-4}$, $P_T < 1 \times 10^{-5}$, $P_T < 1 \times 10^{-6}$, $P_T < 1 \times 10^{-7}$, $P_T < 1 \times 10^{-8}$, $P_T < 1 \times 10^{-9}$ and $P_T < 1 \times 10^{-10}$.

Image acquisition

Participants were scanned on eight 3.0 Tesla Magnetic Resonance Scanners. Specifically, four sites used Siemens Trim 3T scanners and the other four sites used GE SIGNA 3T scanners. No hardware or system upgrade was carried out on any of the scanners during the scanning period. A high-resolution, three-dimensional, T1-weighted brain volume (BRAVO) MRI sequence was performed using a protocol with a matrix size of 256×256, resolution of 1×1 mm^2, inversion time of 1100 ms and slice thickness of 1 mm. A total of 192 sagittal slices were acquired on the Siemens scanners and 188 sagittal slices on the GE scanners. A total of 820
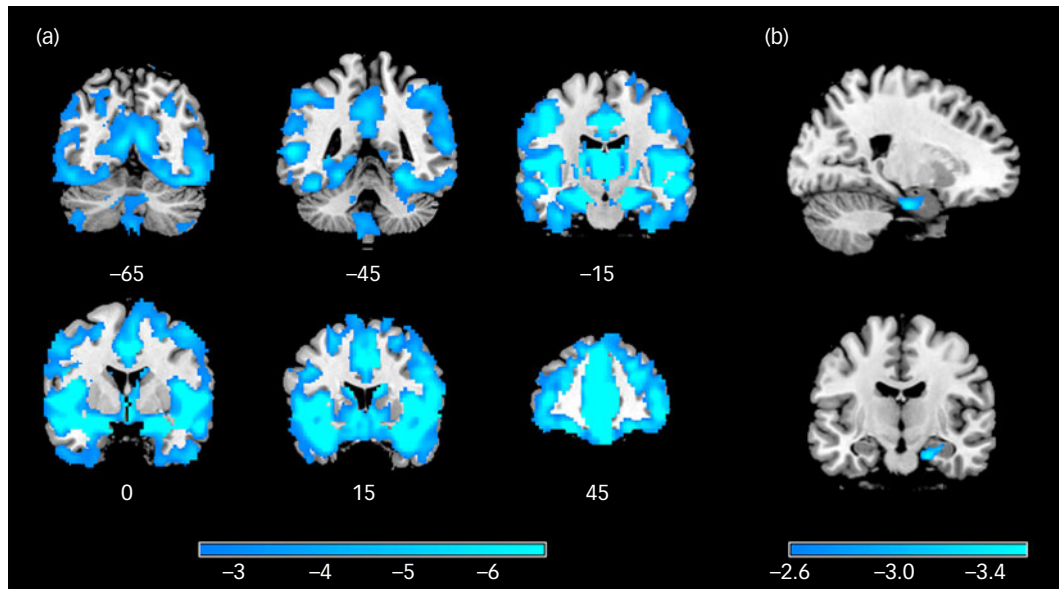
Fig. 1 Association between the schizophrenia polygenic risk score (PGRS) and impaired grey matter volume (GMV). (a) A two-sample *t*-test of the GMV between schizophrenia and healthy control groups with AlphaSim correction (single voxel $P < 0.005$, the corrected threshold $P < 0.05$ and cluster size threshold >161 voxels), the negative *t*-value representing the GMV of this region was significantly decreased in people with schizophrenia. (b) Multiple regression analysis testing the association between PGRS and disrupted GMV with AlphaSim correction (single voxel $P < 0.005$, the corrected threshold $P < 0.05$ and cluster size threshold >132 voxels).

of the original participants, including 409 people with schizophrenia (209 males, mean age 27.78 ± 6.54, age range 17.50–45.41) and 411 healthy controls (197 males, mean age 27.88 ± 6.00, age range 17.75–44.58) had sufficient resting-state functional imaging data and were thus included for further imaging analyses. Resting-state functional imaging was performed using a single-shot, gradient-echo, echo-planar imaging (EPI) sequence that was sensitive to blood oxygen level-dependent contrast with the following parameters: repetition time 2000 ms, echo time 30 ms, flip angle 90°, matrix size 64×64, resolution of axial slice 3.4375×3.4375 mm^2, slice thickness 4 mm, gap between slices 0.6 mm. Only participants from one site had a 6-minute scan (180 time points), whereas the participants from the other sites had an 8-minute scan (240 time points).

Image processing

During the calculation of the voxel-based morphometry (VBM), T1 images were processed using MATLAB for Windows with SPM8 (https://www.fil.ion.ucl.ac.uk/spm/software/spm8/) implemented in the VBM Toolbox 8 (http://dbm.neuro.uni-jena.de/vbm/). Except for applying the affine regularisation according to the International Consortium for Brain Mapping template for East Asian brains, we mainly applied standard routines and default parameters. The T1 images were finally segmented into grey matter, white matter and cerebrospinal fluid and then the grey matter images were smoothed with a 6 mm full width at half maximum isotropic Gaussian kernel.

The data-set was screened for artefacts, head motion (translation >3 mm or degree >3°), registration and normalisation quality. We discarded the first 10 time points of the EPI images. The preprocessing steps of the EPI images included: (a) slice timing correction; (b) head motion correction; (c) rigid-body registration of the T1 image to the EPI mean image; (d) spatial normalization of EPI images to Montreal Neurological Institute (MNI) standard space by using the T1 image and subsequent resampling to $3 \times 3 \times 3$ mm^3; (f) removing the noise from whole brain signals,

head motions and linear trends; and (g) smoothing with a 6 mm Gaussian kernel. All the processes were performed using a MATLAB-based pipeline toolbox BRANT for Windows (https://github.com/kbxu/brant) in which slice timing, realign, co-register, normalise and smooth are functions called from SPM.

For the preprocessed EPI images, the voxel-wise functional connectivity to the whole brain from a specific region of interest was also computed with the BRANT toolbox. The functional connectivity map for each individual was obtained by computing the Pearson's correlation coefficient between the average time series in the region of interest and all voxels in the brain.

Statistical analysis

We performed a voxel-wise, two-sample *t*-test to measure the difference in the GMV between the people with schizophrenia and healthy controls across the whole brain, including age, gender and sites as covariates. Because the participants were from the same population, we did not include the principal components from population stratification as covariates. We focused on whether the impaired GMV was influenced by polygenic risk for schizophrenia, so we defined the regions that were significantly different between the two groups as a mask and then used a voxel-wise multiple regression model to investigate the association between the schizophrenia PGRS and the GMV. Age, gender, sites and diagnosis were again added as covariates. Next, the observed region was extracted and defined as the region of interest and the functional connectivity from the region of interest to the whole brain was computed. Similar statistical methods were applied to the functional connectivity analysis, including a two-sample *t*-test and a multiple regression model. We used SPM to complete the above analyses, and the multiple comparisons were corrected by the AlphaSim method using the Resting-State fMRI Data Analysis Toolkit for Windows (REST; http://restfmri.net/forum). Finally, a Pearson's correlation analysis was performed to test the association of the PANSS scores with the PGRS-related GMV and functional connectivity in the people with schizophrenia with age, gender and sites as covariates.
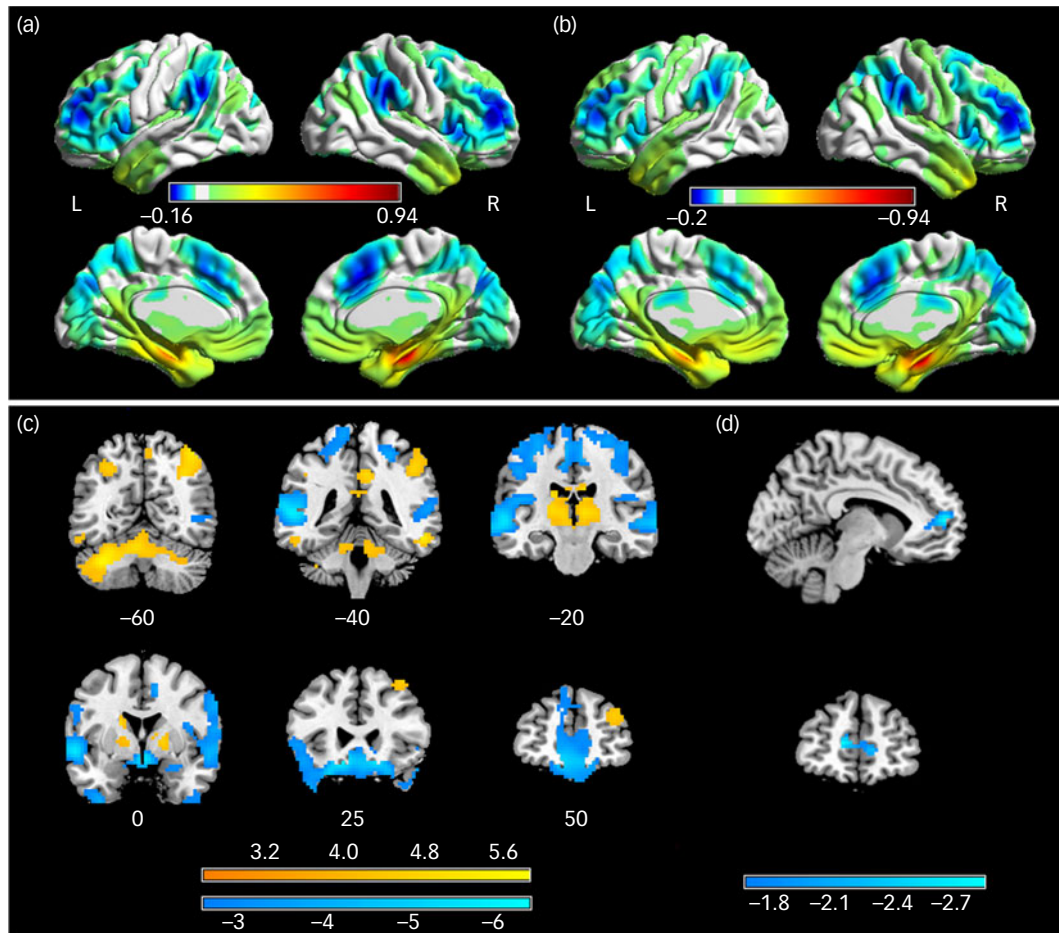
Fig. 2 Association between the schizophrenia polygenic risk score (PGRS) and disrupted functional connectivity. (a) Mean graph of the functional connectivity between the hippocampus and the whole brain in people with schizophrenia. (b) Mean graph of the functional connectivity between the hippocampus and the whole brain in the healthy control group. (c) Two-sample t-test results for the functional connectivity in the schizophrenia and healthy control groups with AlphaSim correction (single voxel $P < 0.005$, corrected threshold $P < 0.05$ and cluster size threshold >21 voxels), the negative t-value representing the functional connectivity was decreased in the people with schizophrenia and increased in the healthy controls. (d) Multiple regression analysis testing the association between the PGRS and the disrupted functional connectivity with AlphaSim correction (single voxel $P < 0.01$, the corrected threshold $P < 0.05$ and cluster size threshold >56 voxels). L, left; R, right.

Similarly, as with all the samples (schizophrenia group and healthy control group combined), we obtained the Pearson's correlation of the PGRS-related GMV and functional connectivity with the cognitive abilities, using age, gender, sites and diagnosis as covariates.

Results

PGRS differences between people with schizophrenia and healthy controls

The participants included 509 people with schizophrenia (275 males, mean age 27.78 ± 6.48, age range 17.50–45.41) and 502 healthy controls (255 males, mean age 27.69 ± 5.91, age range 17.75–44.58) (Table 1). We compared 17 PGRSs between the schizophrenia group and the healthy control group and found that the PGRS in the people with schizophrenia was significantly higher than that in the healthy controls under each threshold. In addition, the PGRS that was computed with a threshold of 0.1 showed the most significant difference between these two groups (Supplementary Fig. 1 available at https://doi.org/10.1192/bjp.2019.127 and Table 1). Therefore, this PGRS was then used in the rest of the analyses

because it best explained the difference in genetic architecture between the patients and the controls.

Association between the schizophrenia PGRS and disrupted GMV

We first investigated the difference in GMV between the people with schizophrenia and the healthy controls. We found decreased GMV in many brain regions, including the thalamus, frontal lobe, cingulate cortex, basal ganglia and hippocampus (Fig. 1a), but observed no increased GMV in the patients compared with the healthy controls. We then analysed the association between the PGRS and the GMV in the brain regions that showed significant case–control differences in GMV and found that a smaller right hippocampal GMV (peak voxel MNI coordinate: $x = 21$, $y = −13$, $z = −24$) was significantly correlated with a higher PGRS (Fig. 1b).

Association between the schizophrenia PGRS and disrupted functional connectivity

We selected the right hippocampus as the region of interest to calculate each individual's hippocampal functional connectivity to the
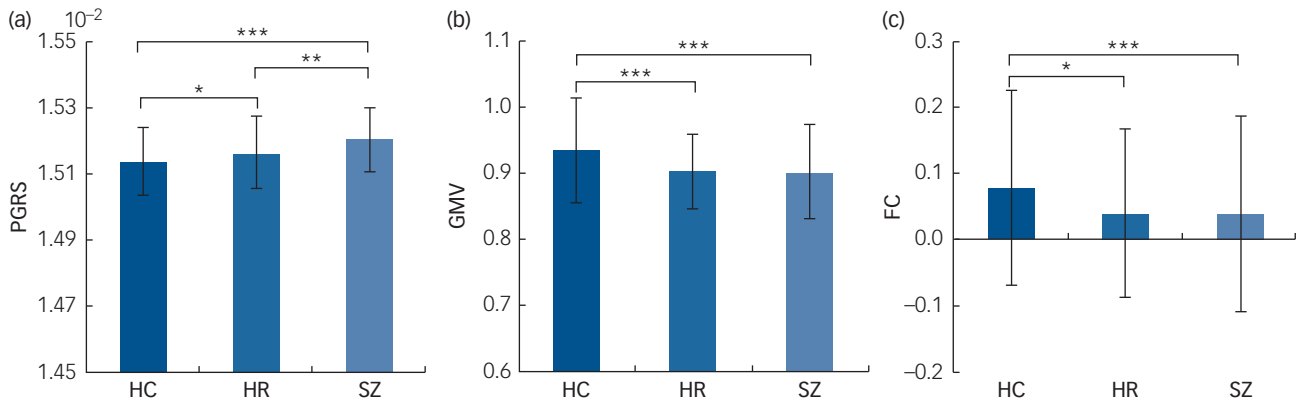
Fig. 3 Polygenic risk score (PGRS), right hippocampal grey matter volume (GMV) and hippocampus–medial prefrontal cortex (mPFC) functional connectivity in the healthy control (HC) group, unaffected first-degree relatives (high-risk individuals, HR) and people with schizophrenia (SZ). (a) Distribution of the PGRS. (b) Distribution of the right hippocampal GMV. (c) Distribution of the hippocampus–mPFC functional connectivity (FC). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

whole brain and found similar functional connectivity patterns in the schizophrenia group and the healthy controls (Fig. 2a and Fig. 1b). Using a two-sample t-test with AlphaSim correction, the functional connectivities from the hippocampus to the thalamus, middle/superior frontal cortex, precuneus and middle temporal cortex were significantly stronger in the patients than in the controls. Conversely, the functional connectivities to the prefrontal cortex, superior temporal cortex, insula lobe and postcentral/precentral gyrus were significantly weaker in the patients (Fig. 2c). We then analysed the association between the PGRS and the disrupted functional connectivities and found a relatively weak correlation between the PGRS and the hippocampus–left/right medial prefrontal cortex (mPFC, peak voxel MNI coordinate: $x = -12$, $y = 51$, $z = 9$) functional connectivity with AlphaSim correction (Fig. 2d).

PGRS, hippocampal GMV and hippocampus–mPFC functional connectivity in unaffected first-degree relatives

To further investigate whether the PGRS-related brain measures reflect fundamental neural circuits that are influenced by genetic factors and could be regarded as plausible biomarkers for the early identification of schizophrenia, we compared the values of the PGRS, hippocampal GMV and hippocampus–left/right mPFC functional connectivity of unaffected first-degree relatives with the healthy control group and the people with schizophrenia, using analysis of variance (Fig. 3). We found that the PGRS ($P_T < 0.1$) for the unaffected first-degree relatives was significantly greater than the PGRS for the healthy control group and considerably smaller than the PGRS for the people with schizophrenia (Fig. 3a). In addition, we found that the hippocampal GMV and hippocampus–mPFC functional connectivity in the unaffected first-degree relatives were slightly greater than those in the people with schizophrenia, and both of them were significantly smaller than those in the healthy control group (Fig. 3, b and c). However, we did not find the significant association of the PGRS for schizophrenia with the hippocampal GMV and hippocampus–mPFC functional connectivity in the unaffected first-degree relatives.

Discussion

In this study we found that the PGRS in the people with schizophrenia was significantly greater than that in the controls. Our imaging

genetic analysis then revealed a genetic effect of the PGRS on the impaired hippocampal GMV and hippocampus–mPFC functional connectivity in a large sample of Han Chinese participants that included both the schizophrenia and healthy control groups. Specifically, the PGRS showed a significantly negative correlation with the hippocampal GMV and hippocampus–mPFC functional connectivity, both of which were smaller in the people with schizophrenia than in the controls. We further found that these brain measures (including the hippocampal GMV and hippocampus–mPFC functional connectivity) showed similar changes in unaffected first-degree relatives as in patients. These findings suggested that the genetically influenced hippocampal GMV and hippocampus–mPFC functional connectivity might be important biomarkers for the early diagnosis of schizophrenia.

We found that, regardless of which threshold was chosen, the PGRS in the schizophrenia group was significantly greater than that in the healthy control group, a finding which is consistent with a previous study.[19] As a measure of cumulative genetic risk, the finding confirmed that the PGRSs successfully captured the difference in genetic structure between the patients and the controls. Moreover, the threshold in this study was chosen to be 0.1 because it best explained the case–control difference in the genetic structure according to our results from the two-sample t-test (Supplementary Fig. 1). This was also in line with previous studies which reported that the thresholds 0.5, 0.1 and 0.05 could maximally capture the heritability of schizophrenia.[1,20,21] In addition, the computation of our PGRSs was based on a meta-analysis of Chinese GWAS samples and Psychiatry Genomics Consortium (PGC2) GWAS samples from Li and colleagues.[12] In fact, they have conducted polygenic scoring analyses to predict the case–control status for Chinese participants and found the PGRS derived from the combined data-set (Chinese plus PGC2) explained a larger proportion of the variance than the data-set with only PGC2 or Chinese samples. The limited sample size may reduce the statistical power of genome-wide meta-analysis using only Chinese samples.

By imaging genetic analyses, we found that the higher PGRS was associated with decreased right hippocampal GMV, which was found to be significantly smaller in people with schizophrenia than in healthy controls. In fact, we found that the bilateral hippocampus and other regions included in the default and salience networks showed impaired GMV in the people with schizophrenia, in line with many previous studies.[22,23] Among these regions, we only found that the right hippocampal GMV had a significantly negative

correlation with the PGRS. Therefore, we speculated that the impaired right hippocampal GMV is influenced by genetic factors. Among the limited studies about the associations between schizophrenia-related genetic variants and volume measures, to the best of our knowledge only hippocampal volume was found to be genetically influenced.[24] One recent study reported they did not find overlapping evidence of genetic variation between schizophrenia and eight subcortical volume measures, including the hippocampus,[25] but they still found a negative tendency for a genetic correlation between hippocampal volume and schizophrenia ($P = 0.068$).[25] Furthermore, the PGRS in our study was computed using a meta-analysis of previously unused Chinese GWAS samples and PGC2 GWAS samples,[12] and the further analysis was also based on a sample of Chinese Han participants. In addition, unlike the research of Franke and colleagues, which considered the whole hippocampal volume,[25] we performed a voxel-wise regression analysis and found a subfield of the right hippocampus that correlated with the schizophrenia PGRS. This was also in line with a previous study that found that not the total hippocampal volume but a subfield volume was influenced by the schizophrenia PGRS.[26] We think that the slightly inconsistent results may be due to differences in ethnicity and methodologies. We further tested the association between impaired hippocampal GMV and cognitive performance and the PANSS scores (Supplementary Table 2). We found that a smaller right hippocampal GMV was significantly associated with a worse SDMT performance ($r = 0.067$, one-tailed $P = 0.019$) and a greater PANSS negative score ($r = -0.083$, one-tailed $P = 0.032$).

We further observed that the hippocampus–mPFC functional connectivity was reduced in people with schizophrenia and that the lower connectivity was correlated with a higher PGRS. Previous studies have found impaired activity and structure of the mPFC in schizophrenia.[32] A study using an animal model reported that alteration of the functional connectivity between the hippocampus and the mPFC possibly contributed to schizophrenia-like symptoms.[33] Moreover, deficits in overnight memory consolidation in schizophrenia were found to be associated with hippocampus–mPFC connectivity.[34] Most importantly, the hippocampus and mPFC are two major hubs in the default mode network, a brain system that is reported to modulate internally directed thought, watchfulness and maintenance of self-perception.[35] This network has attracted considerable interest in neuropsychiatric disorders, including schizophrenia.[36] However, until now, no study has revealed that the hippocampus–mPFC functional connectivity was related to the schizophrenia polygenic information. Some genetic variants associated with schizophrenia, such as the glutamate–cysteine ligase catalytic gene,[37] neuregulen-1/-2[38] and catechol O-methyltransferase messenger RNA[38] have been found to influence mPFC phenotypes such as glutathione metabolism dysregulation, extracellular dopamine dysregulation and reduced grey matter concentration. To some extent, these studies support our conclusion that the connectivity from the mPFC is influenced by a schizophrenia genetic mechanism. In addition, we also tested the association between the hippocampus–mPFC functional connectivity and the cognitive abilities and PANSS scores, but found no significant association (Supplementary Table 2).

Our investigation of unaffected individuals at high familial risk of developing schizophrenia found that their identified brain measures were similar to those in the patients with schizophrenia and that their PGRSs fell between the schizophrenia and healthy control groups. These results further supported our finding that the hippocampal GMV and hippocampus–mPFC functional connectivity are important imaging endophenotypes, findings which could help in the early identification and diagnosis of schizophrenia. Currently, schizophrenia is primarily diagnosed using clinical

symptoms, and no reliable biological marker has been identified to improve early detection.[39] Our study in unaffected first-degree relatives seems to provide complementary evidence for PGRS-related biomarkers and may help to link vulnerability genes to clinical syndromes. In line with our findings, many previous studies found that unaffected first-degree relatives show similar but milder neuroimaging abnormalities compared with schizophrenia patients[40] and a higher PGRS than healthy controls.[39] But we did not find the significant association between PGRS and the observed neuroimaging abnormalities in unaffected first-degree relatives. The main reason for this may be that these first-degree relatives are of a relatively older age and consist of a smaller sample size. Moreover, some of them are siblings of the patients and others are parents of the patients. Overall, our current findings, accompanied by the supporting evidence from unaffected first-degree relatives, indicate that hippocampal GMV and hippocampus–mPFC functional connectivity could be traits or vulnerability markers for schizophrenia.

Although our study reported plausible schizophrenia polygenic effects on hippocampal GMV and hippocampus–mPFC functional connectivity, several issues still need to be considered and addressed in future studies. First, although the sample size of our study was quite large, the findings still need to be replicated in independent samples. Second, the aim of our study was to identify plausible endophenotypes, so a relatively weak multiple comparison correction method, AlphaSim, was used. However, the results in a cohort of unaffected first-degree relatives further confirmed the clinical significance of our findings. Third, the present study was based on participants of Han Chinese ancestry, so the findings may need to be tested in other ethnic populations. Finally, because the majority of patients included in our study were taking psychotropic drugs during MRI scanning and we had no lifestyle records, the current results did not control for the potential effects of these drugs on people with schizophrenia. To examine whether our observed neuroimaging measures were caused by drug exposure unique to the patient group, we carefully consulted every patient's medication record and found that only 22 of all patients were first-episode drug naive. We then compared the 22 first-episode, drug-naive patients with those of 22 randomly selected healthy controls for 1000 permutation tests and found hippocampal GMV and hippocampus–mPFC functional connectivity in first-episode, drug-naïve patients were significantly smaller than those in healthy controls ($P = 0.0186$ and 0.0363, respectively). This result indicated that our current finding may be potential biomarkers for schizophrenia. However, because of the small sample size of first-episode, drug-naive patients and no exclusion of the influence of lifestyle factors, future study is needed to further validate our findings.

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Supplementary material

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