# DNA hyper-methylation associated with schizophrenia may lead to breakdown of self-tolerance

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**Article**

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## Abstract

Environmental stressors have effects on the genomic DNA methylation patterns of B lymphocytes in schizophrenia (SCZ), which may therefore perturb the immune homeostasis and trigger autoimmune responses. This study aimed to investigate the global genomic DNA methylation differences in remitters of SCZ (RSCZ) and non-remitters of SCZ (NRSCZ), further their effects on autoimmune responses of target genes. A total of 2722 Chinese Han origin subjects were recruited, including a follow-up cohort and a cross-sectional cohort. We found a DMS of cg14341177 in SCZ which inhibit the mRNA alternative splicing of *BICD2*, further leading to increased plasma anti-BICD2 IgG autoantibody levels. The levels of cg14341177 methylation and anti-BICD2 IgG decreased significantly in the endpoint samples of RSCZ, but not in NRSCZ. There are strong positive correlations between cg14341177 methylation, anti-BICD2 IgG, and PANSS scores. These data suggest that cg14341177 methylation and anti-BICD2 IgG levels may potentially serve as useful biomarkers.

## Introduction

Schizophrenia (SCZ) is a severe debilitating disorder, affecting 0.7% of the population worldwide [1]. The etiology of SCZ is complicated, wherein both genetic and environmental factors play important roles in its development, leading to high heterogeneity in patient populations.

Recent studies have highlighted a neuro-immune mechanism in the pathogenesis of SCZ, especially with regard to the chronic neuro-inflammatory responses. The literatures on the genome-wide association study (GWAS) in the last five years confirmed hundreds of genetic loci that are significantly associated with the risk of SCZ [2, 3]. The most remarkable genetic association signal is the human leukocyte antigen (HLA) region in the short arm of chromosome 6, which exhibits an autoimmune role in SCZ [2]. The spatiotemporal expression of SCZ-GWAS candidate genes is highly associated with the key time point in neurodevelopment processes. Certain genes are highly expressed in brain tissues and B lymphocytes, which further support the psycho-neuro-immunological hypothesis in SCZ [2, 3]. A study pertaining to SCZ-GWAS candidate genes reported altered autoantibody levels in first-episode schizophrenia (FES) patients [4]. SCZ-GWAS gene-derived autoantigen treatments in cultured B lymphocytes enhance the proportions of CD83 + cells and apoptotic B cells, which suggests a tolerance breakdown mechanism in SCZ [5].

However, the heterogeneous spectrum of SCZ and divergent responses to antipsychotic medications cannot be solely explained through genetics, but also requires environmental factors to be taken into consideration [6]. Epigenetics can better explain the gene-environment interactions for the disease [7, 8]. The different responses to antipsychotic treatment in patients with SCZ are assumed because of DNA methylation and histone modification patterns [7, 9]. Several studies that focused on differential DNA methylation patterns in SCZ [9, 10, 11, 12, 13], and have identified differentially regulated DNA methylation biomarkers involved in divergent antipsychotic medication responses [7]. In rodent models, treatment with antipsychotic drugs can induce changes in DNA methylation of neuroregulatory genes such as dopamine receptor family genes, cadherin family genes, and neurotrophic factor genes, and thus, affect their expression profiles and animal behavioral phenotypes[14, 15, 16]. Antipsychotic medication reduces chronic inflammation in patients with SCZ by modifying the secretion of inflammatory cytokines and activation of peripheral immune cells [17, 18, 19].

Therefore, we aimed to determine the effect of DNA methylation changes on self-tolerance breakdown in SCZ. Goals of the present study included (1) analyzing the genome-wide DNA methylation profiles in SCZ case-control cohorts to identify immune-related DMS, (ii) measuring the autoantibody levels encoded by causal candidate genes, and (iii) analyzing the correlation between these biomarkers and the clinical psychopathology of patients with SCZ.

## Material And Methods

# Experimental Design

A multi-stage, case-control study and a follow up plan were designed to investigate whether differences in DNA methylation in SCZ affect autoimmune responses and correlate with clinical symptoms (Figure S1).

# Sample Description

A total of 2722 Chinese Han origin subjects were recruited in this study (2005–2011), including 1390 paranoid schizophrenia patients according to ICD-10 (F20.0) and 1332 controls (Table 1). All the patients were diagnosed by two consultant psychiatrists following a structured interview assessment process. The participants met all the inclusion criteria, without any exclusion criteria (Table S1). All participants provided written informed consent, and the study was approved by the local ethics committees and conformed to the requirements of the Declaration of Helsinki.

Table 1
Demographic and clinical features of the participants.

| Demographic information | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Discovery Cohort | | | | | Replication Cohort | | | | | Validation Cohort | | | | |
| | Age, y | SD | Male | Female | | Age, y | SD | Male | Female | | Age, y | SD | Male | Female |
| RSCZ (n = 40) | 25.08 | 7.04 | 20 | 20 | RSCZ (n = 64) | 25.48 | 7.67 | 31 | 33 | SCZ (n = 1230) | 39.58 | 13.61 | 698 | 532 |
| NRSCZ (n = 40) | 24.23 | 8.43 | 18 | 22 | NRSCZ (n = 16) | 21.06 | 4.43 | 5 | 11 | CTL (n = 1208) | 37.39 | 13.61 | 544 | 644 |
| CTL (n = 40) | 25.51 | 5.39 | 20 | 20 | CTL (n = 84) | 28.77 | 8.58 | 30 | 54 | | | | | |
| PANSS scores | | | | | | | | | | | | | | |
| Baseline | Discovery Cohort | | | | | Replication Cohort | | | | | Meta | | | |
| | RSCZ | SD | NRSCZ | SD | P value | RSCZ | SD | NRSCZ | SD | P value | RSCZ | SD | NRSCZ | SD |
| PANSS Positive Subscales | 23.00 | 5.32 | 23.20 | 4.57 | 0.012 | 22.75 | 5.13 | 22.00 | 4.47 | 0.122 | 23.35 | 5.04 | 22.52 | 4.19 |
| PANSS Negative Subscales | 21.08 | 7.54 | 23.23 | 6.75 | 0.079 | 21.06 | 6.27 | 18.19 | 6.72 | 0.015 | 21.39 | 6.79 | 22.10 | 6.92 |
| General Psychopathology Subscales | 42.05 | 6.90 | 41.25 | 7.27 | 0.992 | 42.34 | 6.81 | 38.38 | 5.77 | 0.009 | 42.76 | 6.97 | 40.67 | 6.41 |
| PANSS Total | 86.03 | 14.04 | 87.68 | 14.60 | 0.382 | 86.23 | 12.34 | 78.56 | 10.15 | 0.001 | 87.46 | 13.07 | 85.29 | 12.47 |
| Endpoint | Discovery Cohort | | | | | Replication Cohort | | | | | Meta | | | |
| | RSCZ | SD | NRSCZ | SD | P value | RSCZ | SD | NRSCZ | SD | P value | RSCZ | SD | NRSCZ | SD |
| PANSS Positive Subscales | 7.15 | 0.48 | 9.70 | 4.40 | 0.501 | 7.16 | 0.51 | 9.44 | 2.73 | 7.30E-06 | 7.12 | 0.45 | 9.54 | 3.52 |
| PANSS Negative Subscales | 9.03 | 2.71 | 16.03 | 5.61 | 4.87E-11 | 8.78 | 1.98 | 14.36 | 4.33 | 1.64E-08 | 8.89 | 2.37 | 15.88 | 5.16 |
| General Psychopathology Subscales | 18.60 | 2.31 | 22.48 | 6.11 | 4.10E-04 | 18.08 | 1.79 | 22.38 | 4.01 | 1.07E-06 | 18.24 | 2.03 | 22.35 | 5.36 |
| PANSS Total | 34.78 | 4.38 | 48.20 | 13.12 | 4.98E-09 | 34.03 | 3.08 | 46.18 | 7.33 | 8.65E-13 | 34.25 | 3.66 | 47.77 | 10.78 |
| Abbreviations: | | | | | | | | | | | | | | |
| RSCZ, remitter of schizophrenia; NRSCZ, non-remitter of schizophrenia; CTL, control; SCZ, schizophrenia; PANSS, positive and negative syndrome scale; SD, s deviations. | | | | | | | | | | | | | | |

A total of 160 FES patients derived from a previous trial (ClinicalTrials, identifier NCT01057849) underwent a one year follow-up study under treatment with atypical antipsychotics, including risperidone, aripiprazole, and olanzapine (2008–2011) [20]. The definition of remission was the simultaneous attainment of symptomatic and duration criteria according to the positive and negative syndrome scale (PANSS), as proposed by the Remission in Schizophrenia Working Group [21].

## Genome-Wide DNA Methylation Profiling

In total, 200 samples from the discovery cohort were hybridized with the Illumina Human MethylationEPIC BeadChip arrays to profile the genome-wide methylation levels at more than 850,000 CpG sites. For data quality control, any technically unreliable probes were removed, including cross-hybridizing probes, probes located on sex chromosomes and SNPs overlapping probes. Methylation levels for each site were calculated using the R minfi package (version 1.28.4) and adjusted *M* values were used to run all statistical analysis[22]. However, $\beta$ values were used for ease of clarity and interpretation. The R sva package (version 3.30.1) was performed to correct for batch effects [23]. For extraction of DMS, we fitted a linear regression model for each CpG site: *M* value + age + error, and next applied an empirical Bayes smoothing to the standard errors using the R Bioconductor limma package (version 3.38.3) [24]. DMS were extracted using a threshold of FDR-adjusted *P* value < 0.01 and the mean $\beta$ value difference $|\triangle\beta|$ >5%. GO functional enrichment analysis of genes harboring DMS was performed using the R clusterProfiler package (v3.10.1) with a FDR-adjusted *P* value < 0.05 for statistical significance.

## Sequenom MassARRAY DNA quantitative methylation assay

A Sequenom MassARRAY platform was used to perform the quantitative methylation analysis of candidate DMS. The PCR primers listed in Table S2 were designed using Sequenom Epidesigner (http://www.epidesigner.com). For each reverse primer, an additional T7 promoter tag for in vivo transcription was added, whereas a 10 mer tag on the forward primer was used to adjust melting temperature differences. After PCR amplication, in vitro RNA transcription was performed on the reverse strand and then digested via base-specific cleavage. Mass spectra were obtained via MassARRAY Compact MALDI-TOF (Sequenom, CA, USA) and their methylation ratios were generated using Epityper software (Sequenom, CA, USA).

## Detection of BICD2 transcripts expression levels

A taqman-probe based multiplex-qRT-PCR method was used for detecting the *BICD2* transcript levels. The primers and probes listed in Table S2 were designed using Primer Express™ Software v3.0.1 (Applied Biosystems, CA, USA), and their location were marked in the Figure S2.

In brief, for total *BICD2* quantification, the primer and probe sets of *BICD2* total and *GAPDH* were added in a reaction, which occupied HEX channel and FAM channel, respectively. Similarly, *BICD2* total & *BICD2* isoform 1 (NM_001003800) and *BICD2* total & *BICD2* isoform 2 (NM_015250) combinations were used for each transcript, respectively. Amplifications were performed in a Roche LightCycler® 96 system (Roche, IN, USA), using the default cycling conditions for 45 cycles. All samples were performed in triple independently biological repeats, and the results were calculated by $2^{-(\triangle Ct\_SCZ - \triangle Ct\_CTL)}$ formula.

## Detection of plasma anti-BICD2 IgG antibody levels

The computational prediction of the HLA-II epitopes was performed in the Immune Epitope Database (25) and found a 24 amino acid linear peptide (SDRAEGTGLANQVFCSEKHSIYCD) on the C-terminal tail of BICD2 isoform 1 protein as a potential autoantigen (Figure S3).

A previously reported in-house ELISA assay was optimized for plasma anti-BICD2 IgG antibodies detection [4]. The 24mer linear epitope was synthesized by solid-phase chemistry with a purity of > 95% (Bootech, Shanghai, China), and dissolved in 67% acetic acid to a concentration of 5 mg/mL and stored at -20°C. The stock solution was diluted in coating buffer (0.1 M phosphate buffer, 0.15 M NaCl, 10 mM EDTA and pH 7.2) to a concentration of 20 μg/mL as working solution. A maleimide activated ELISA plate (Corning, ME, USA) was coated with 100 μL/well of working solution and incubated 2 h at room temperature (RT). The plates were washed 3 times using 200 μL/well of wash buffer 1 (0.1 M phosphate buffer, 0.15 M NaCl and pH 7.2), and blocked using 10 μg/mL cysteine in coating buffer for 1 h at RT. Then washed twice using wash buffer 1 and dried at 60°C for 3 h. Seal the dried plates and stored at 4°C until use. The coated plates needed to be rehydrated before use by washed twice with 200 μL/well of wash buffer2 (0.01 M phosphate-buffered saline (PBS) containing 0.1% Tween-20). Plasma samples as well as positive control (PC) and quality control (QC) were diluted 1:100 in assay buffer (0.01 M PBS containing 0.5% bovine serum albumin) and loaded 50 μL/well, while 50 μL of assay buffer was added to each negative control (NC) well. All samples were performed in twice independent biological repeats. The plates were incubated at RT for 2 h and then washed 3 times with wash buffer 2.

A peroxidase-conjugated goat anti-human IgG Fc secondary antibody (ab98624, Abcam, Cambridge, UK) was diluted 1:50,000 in assay buffer loaded 100 μL/well and incubated for 2 h at 4°C and washed 3 times. Finally, loaded 100 μL/well of 3, 3', 5, 5'-tetramethylbenzidine (TMB, PR1210, Solarbio, Beijing, China) and incubated in the dark for 20 min before 50 μL/well of the stop solution (12% sulphuric acid) was added. The optical density (OD) of each well was then measured within 10 min with a plate reader at 450 nm with a reference wavelength of 620 nm. The specific binding ratio (SBR) was calculating for each sample using the following formula:

$SBR= (OD_{sample} - OD_{NC})/ (OD_{PC} - OD_{NC})$

## Statistical Analysis

Results are presented as mean with SD unless otherwise stated. A univariate linear model was used to compare the differences of demographic, PANSS, DNA methylation, and anti-BICD2 IgG levels between groups, which considered age and gender as covariates. Paired t-test was used to compare the differences between baseline and endpoint samples. Pearson's correlation analysis was used to test the correlation in cg14341177 methylation, anti-BICD2 IgG, and PANSS scores. The coefficient of variation (CV) was used to represent an inter-assay deviation using a quality control (QC) sample, which were randomly collected from 50 healthy subjects, pooled, and tested on every 96-well microtiter plate. A $P$ value < 0.05 was considered to be statistically significant in this study. Analyses were performed on the IBM SPSS Statistics software (Version 20.0., IBM Corp., Armonk, NY, USA) or GraphPad software (Version 6.01, San Diego, CA, USA).

### Results

## Demographic and clinical characteristics of the subjects

In the 160 SCZ patients who completed the 1-year of antipsychotic treatment, there was no difference between remitters of schizophrenia (RSCZ) and non-remitters of schizophrenia (NRSCZ) in terms of baseline demographic and clinical characteristics. At the endpoint of 1-year follow-up, 56 NRSCZ showed higher PANSS scores than RSCZ (Table 1). The remaining 1230 SCZ patients were diagnosed in acute schizophrenic episode. All control subjects (CTL) have no mental illness or family history.

We thus divided these subjects into a discovery cohort, a replication cohort and a validation cohort. The discovery cohort contained 40 RSCZ, 40 NRSCZ and 40 CTL subjects well matched in age and sex. The replication cohort contained the remaining 64 RSCZ and 16 NRSCZ, as well as 84 CTL subjects. The enlarged cross-sectional validation cohort contained 1230 SCZ and 1208 CTL subjects (Table 1).

## Genomic differences in DNA methylation profiles in RSCZ and NRSCZ

We firstly analyzed the genome-wide DNA methylation variation at > 850,000 CpG sites in the discovery cohort. After normalization and filtering of data, we retained a final dataset of 682,651 CpG sites in the 120 subjects. Principal component (PC) analysis showed a clear distinction between RSCZ and NRSCZ from CTL along the first two PCs (Fig. 1A). However, the difference was less between baseline and endpoint DNA samples, indicating that the antipsychotic medications did not cause global DNA methylation changes. A total of 53,659 DMS were identified and mapped to 11,749 genes at a 1% FDR and a mean difference (delta $\beta$) > 5% (Table S3).

The genomic distribution of DMS was quite different between RSCZ and NRSCZ groups. In RSCZ, hyper-methylated DMS were enriched in 5'-UTR, gene body, 3'-UTR and enhancer region, while hypo-methylated DMS were enriched in TSS200, 5'-UTR, 1st exon and promoter. In NRSCZ, hyper-methylated DMS were enriched in TSS200, 5'-UTR, 1st exon and enhancer, but no hypo-methylated DMS enriched region was found (Fig. 1B). There were more state-dependent DMS in the RSCZ group (476 DMS) than the NRSCZ group (17 DMS) when comparing their baseline and endpoint samples (Table S4-5). The cg14341177 was the foremost hyper-methylated DMS in the baseline DNA samples of the RSCZ group (fold change [FC] = 4.171, $P$ = 1.42E-11), and its level decreased in the endpoint DNA samples (FC = -3.397, $P$ = 2.41E-7). A similar state-dependent change was observed in case of the foremost hypo-methylated DMS cg13978347 (Baseline: FC = -2.666, $P$ = 1.59E-6; Endpoint: FC = 2.325, $P$ = 3.02E-4). However, these changes were not found in the NRSCZ group (Fig. 1C, Table S4-5). Gene Ontology (GO) analysis of genes corresponding to DMS also showed different clustered biological processes between the RSCZ and NRSCZ. The top 10 enriched pathways in the RSCZ group were mostly associated with the regulation of T cells and neutrophils; however, those in the NRSCZ group were associated with extracellular matrix, neuronal guidance and synaptic signal transmission (Fig. 1D, Table S6).

## Validation of candidate DMS by quantitative methylation assay

A Sequenom MassARRAY quantitative methylation assay was performed to measure the methylation levels of candidate DMS cg14341177 and cg13978347 in the discovery cohort. There are 4 CpG sites on cg14341177, in which the CpG2 site showed the most significant changes in methylation levels and seemed as the leading CpG site. In the baseline DNA samples, the methylation level of CpG2 was higher in the RSCZ (FC = 1.40, $P$ = 8.338E-11) and NRSCZ group (FC = 1.12, $P$ = 0.027) than those in the CTL group. In the endpoint DNA samples, the methylation level of CpG2 was significantly lower in the RSCZ group (FC = 0.80, $P$ = 3.329E-7) than those in the baseline samples, but not in the NRSCZ group (FC = 1.03, $P$ = 0.210) (Table 2). However, significant changes in the DNA methylation levels of cg13978347 were not observed, and therefore, were not considered for further analysis (Table 2). We got similar results in the replication cohort of 164 independent subjects (64 RSCZ, 16 NRSCZ, and 80 CTL), the hyper-methylation levels of cg14341177 were state-dependent in RSCZ (Baseline: FC = 1.15, $P$ = 3.577E-6; Endpoint: FC = 0.83, $P$ = 3.48E-7) but not in NRSCZ (Table 2). These results were further confirmed in the validation cohort (1230 SCZ vs. 1208 CTL) and were finely reproduced (FC = 1.26, $P$ = 1.896E-145) (Fig. 2).

Table 2
Quantitative methylation assay for candidate DMS.

| Position | CTL | | RSCZ | | | | NRSCZ | | | | RSCZ | | | NRS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SD | Baseline | SD | Endpoint | SD | Baseline | SD | Endpoint | SD | Baseline v.s. CTL[a] | Endpoint v.s. CTL[a] | Baseline v.s. End-Point[b] | Base v.s. CTL |
| **Discovery Cohort** | | | | | | | | | | | | | | |
| **cg14341177** | | | | | | | | | | | | | | |
| **CpG_1** | 0.823 | 0.040 | 0.855 | 0.019 | 0.843 | 0.030 | 0.833 | 0.026 | 0.838 | 0.022 | 4.98E-05 | 0.033 | 0.025 | 0.29 |
| **CpG_2** | 0.200 | 0.045 | 0.280 | 0.045 | 0.225 | 0.040 | 0.223 | 0.047 | 0.231 | 0.047 | 8.34E-11 | 0.009 | 3.33E-07 | 0.02 |
| **CpG_3&4** | 0.830 | 0.033 | 0.840 | 0.050 | 0.825 | 0.068 | 0.834 | 0.028 | 0.844 | 0.032 | 0.361 | 0.723 | 0.324 | 0.61 |
| **DMS** | 0.618 | 0.029 | 0.656 | 0.030 | 0.631 | 0.034 | 0.630 | 0.027 | 0.638 | 0.030 | 1.69E-07 | 0.084 | 3.51E-04 | 0.08 |
| **cg13978347** | | | | | | | | | | | | | | |
| **CpG_1** | 0.735 | 0.087 | 0.744 | 0.056 | 0.735 | 0.083 | 0.730 | 0.070 | 0.749 | 0.112 | 0.599 | 0.627 | 0.986 | 0.70 |
| **CpG_2** | 0.868 | 0.175 | 0.851 | 0.181 | 0.882 | 0.141 | 0.873 | 0.114 | 0.867 | 0.136 | 0.357 | 0.753 | 0.458 | 0.48 |
| **CpG_3** | 0.978 | 0.039 | 0.996 | 0.039 | 0.971 | 0.071 | 0.970 | 0.057 | 0.975 | 0.064 | 0.452 | 0.562 | 0.388 | 0.26 |
| **DMS** | 0.860 | 0.076 | 0.856 | 0.072 | 0.863 | 0.063 | 0.857 | 0.048 | 0.861 | 0.050 | 0.663 | 0.839 | 0.748 | 0.33 |
| **Replication Cohort** | | | | | | | | | | | | | | |
| **cg14341177** | | | | | | | | | | | | | | |
| **CpG_1** | 0.833 | 0.019 | 0.833 | 0.024 | 0.831 | 0.019 | 0.827 | 0.023 | 0.823 | 0.019 | 0.993 | 0.921 | 0.559 | 0.07 |
| **CpG_2** | 0.202 | 0.026 | 0.233 | 0.043 | 0.193 | 0.035 | 0.206 | 0.038 | 0.209 | 0.046 | 3.58E-06 | 0.376 | 3.48E-07 | 0.25 |
| **CpG_3&4** | 0.832 | 0.033 | 0.830 | 0.038 | 0.834 | 0.029 | 0.838 | 0.029 | 0.833 | 0.024 | 0.499 | 0.784 | 0.506 | 0.14 |
| **DMS** | 0.622 | 0.018 | 0.631 | 0.026 | 0.620 | 0.019 | 0.622 | 0.024 | 0.624 | 0.022 | 0.062 | 0.635 | 0.003 | 0.00 |

Abbreviations: CTL, control; RSCZ, remitter of schizophrenia; NRSCZ, non-remitter of schizophrenia, DMS, differential methylation site. Superscripts: a. *P* valu model; b. paired t-test *P* values.

## Hyper-methylation of cg14341177 may inhibit alternative-splicing of BICD2 mRNA in patients with SCZ

The DMS cg14341177 is located on exon 7 of the bicaudal D cargo adaptor 2 (*BICD2)* gene and next to the 3'-end of the alternative splicing region in *BICD2* isoform 1 mRNA (alternative intron 7, I7'), the splicing of which leads to a truncated *BICD2* isoform 2 (Figure S2). A higher level of I7' has been reported in the blood and mortem brain samples in SCZ [25]. We thus speculated that the hyper-methylation in the DMS cg14341177 may affect the alternative splicing events of *BICD2*. We detected *BICD2* mRNA levels in the peripheral blood mononuclear cells (PBMCs) of 155 subjects (59 CTL vs. 96 SCZ) by performing isoform-specific qRT-PCR. No significant changes were found in the levels of total *BICD2* and *BICD2* isoform 2 mRNAs. However, *BICD2* isoform 1 mRNA levels were significantly higher in patients with SCZ (FC = 2.4, $P < 0.0001$) (Fig. 3A). A significantly positive correlation was observed between *BICD2* isoform 1 mRNA and cg14341177 methylation levels (r = 0.4717, $P < 0.0001$), whereas a significant negative correlation was observed between *BICD2* isoform 2 mRNA and cg14341177 methylation levels (r = -0.1867, $P = 0.0349$), respectively (Fig. 3B). These results suggested that the hyper-methylation in the DMS cg14341177 could inhibit the alternative splicing of *BICD2* mRNAs.

## Plasma BICD2 IgG antibody levels increased in patients with SCZ

According to the data obtained from Human Protein Atlas database, BICD2 is widely expressed in the brain and peripheral immune cells, especially in the cortex, hippocampus, amygdala, B lymphocytes, neutrophils, and dendritic cells (Figure S4) [26]. This implies that BICD2 may have some role in the neuroimmunity process. The BICD2 isoform 1 protein has an extra C terminal tail of 31 amino acid residues, which comprises a 24-mer linear autoantigen predicted by IEDB (Figure S3A) [27, 28]. We thus speculated that the inhibition of *BICD2* mRNA splicing by hyper-methylation in the DMS cg14341177 may affect the levels of circulating anti-BICD2 IgG autoantibodies. By performing an in-house ELISA assay against the autoantigen of BICD2 (Figure S3B), plasma anti-BICD2 IgG levels of 1011 subjects (486 CTL vs. 525 SCZ) were measured and significantly elevated anti-BICD2 IgG was detected in SCZ samples (FC = 1.68, $P < 0.0001$) (Fig. 4A). The reproducibility of the ELISA assay was excellent, with an inter-assay coefficient of variation of 9.3% for a total of 31 plates. In the follow-up cohorts, anti-BICD2 IgG levels decreased in the RSCZ endpoint samples (FC = 0.78, $P < 0.0001$), whereas the levels slightly increased in the NRSCZ samples (FC = 1.07, $P = 0.0136$) (Fig. 4B).

## Correlation between cg14341177 DNA methylation, anti-BICD2 IgG, and PANSS scores

The above results showed that the levels of cg14341177 methylation and anti-BICD2 IgG levels can be considered as potential biomarkers for SCZ and its state of remission. Pearson's correlation analysis was performed for all the patients in the follow-up cohorts. In the RSCZ group, a strong positive correlation was observed between cg14341177 methylation and anti-BICD2 IgG levels in the baseline ($r = 0.2762$, $P = 0.0045$), and was most significant in the leading CpG2 ($r = 0.4732$, $P < 0.0001$). A similar correlation was observed when CpG2 and anti-BICD2 IgG levels were compared with PANSS negative subscale scores (CpG2: $r = 0.5052$, $P < 0.0001$; anti-BICD2 IgG: $r = 0.0.5969$, $P < 0.0001$) and PANSS total scores (CpG2: $r = 0.3242$, $P = 0.0008$; anti-BICD2 IgG: $r = 0.3731$, $P < 0.0001$), respectively. However, a non-significant correlation was observed in the NRSCZ group (Fig. 5 and Table S7).

## Discussion

The present study confirmed that extensive differences exist in global DNA methylation profiles between first-episode RSCZ and NRSCZ, as well as the genomic distribution of DMS, and DMS harbored genes and their biological processes. The DMS cg14341177 was the foremost hyper-methylated DMS with decreased methylation levels in the RSCZ group after the administration of antipsychotic medications for one year, but not in the NRSCZ group. Moreover, the hyper-methylated cg14341177 may inhibit the alternative splicing of its harbored gene *BICD2*, resulting in the increase of its longer isoform 1 transcript products. The plasma anti-BICD2 IgG levels were also increased in SCZ, and their change patterns were similar to cg14341177 methylation in the follow-up cohorts. Lastly, the alterations in cg14341177 methylation, plasma anti-BICD2 IgG, and PANSS negative subscale scores were highly correlated in the RSCZ group, and not in the NRSCZ group.

*BICD2* gene is located on 9q22.31, and encodes a conserved cargo adaptor protein required for dynein-mediated transportation. Functionally, BICD2 has been reported to be localized in the Golgi complex and participates in membrane traffic from the Golgi apparatus toward the endoplasmic reticulum (ER) via a coat complex coatomer protein I (COPI)-independent pathway [29]. In mice, the deficiency of *BICD2* affects radial cerebellar migration of granule cells in the developing brain [30]. In humans, coding mutations were also found in spinal muscular atrophy [31, 32]. In PBMCs, *BICD2* is most abundant in naive B lymphocytes (Figure S4). A recent study confirmed that a dynein subunit (DYNLL1) is particularly critical for the development of B-1a lymphocytes, which is a major source of autoantibodies and natural antibodies [33]. Thus the increase of anti-BICD2 IgG levels may disrupt the dynein-mediated cargo transportation in neurons and B lymphocytes, and further breakdown the neuro-immune crosstalk in SCZ. Further investigations are wanted to explore the underlying mechanism by which BICD2 may play a central role in regulating dynein-mediated transport in neuro-immune processes.

Hyperactivity of immune components contributes to the etiology of SCZ to some extent. Previous studies have suggested that increased levels of pro-inflammatory molecules in both acute and chronic SCZ, as well as antipsychotic medications can reduce inflammation in some patients with SCZ [34, 35]. Antipsychotic medications can also reduce disease severity, inflammatory cytokines, and autoantibody levels in autoimmune encephalomyelitis, which has overlapped clinical symptoms with SCZ [36]. This is probably because of the wide distribution of dopaminergic and serotonergic receptors in immune cells, antipsychotic drugs treatment could regulate the differentiation and maturation of immune cells and further their responses [37, 38].We thus speculated that some common regulatory mechanisms may be involved in brain and peripheral lymphocytes in patients with SCZ, and especially those involving autoreactive processes. However, the underlying neuro-immune regulatory mechanisms may vary and more efforts are required.

This study has some limitations. First, the confounding effects of lifestyle-related factors such as smoking, alcohol consumption, and diet in patients were not controlled in this study. Second, the validation cohort subjects were a mix of first-onset and chronic patients with SCZ, as well as drug-free and medication patients. These factors may have interfered with the assay results to some extent. Third, limited clinical information of the subjects was available such as history of autoimmune disorders or hypersensitivities that may have affected our analysis, although the prevalence of autoimmune conditions is no more than 2% in the Chinese population [39]. It would also be useful to test these biomarkers in individuals with bipolar or autism spectrum disorders, or other psychiatric diseases to determine if these abnormalities are limited to SCZ.

## Declarations


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### Author contributions

Q.X and X.Y conceived the project. H.W, Y.Y, C.Z contributed equally to this work. All authors contributed to the experimental design, analysis, and interpretation of results. Study concept and design: H.W, X.Y, Q.X. Acquisition of data: All authors. Clinical data and biological sample collection: Y.Y, F.Y, Z.L, C.W, H.D, J.Z, R.T. Drafting of the manuscript: H.W, X.Y, Q.X. Statistical analysis: H.W, C.Z. Obtained funding: H.W, X.Y, Q.X. Study supervision: H.W, X.Y, Q.X. All authors have reviewed and approved the final paper.

### CONFLICT OF INTEREST

The authors report no financial interests or potential conflicts of interest.

### Additional information

**Supplementary information**

Supplementary Data are available at *Nature Communications* online.

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## Figures

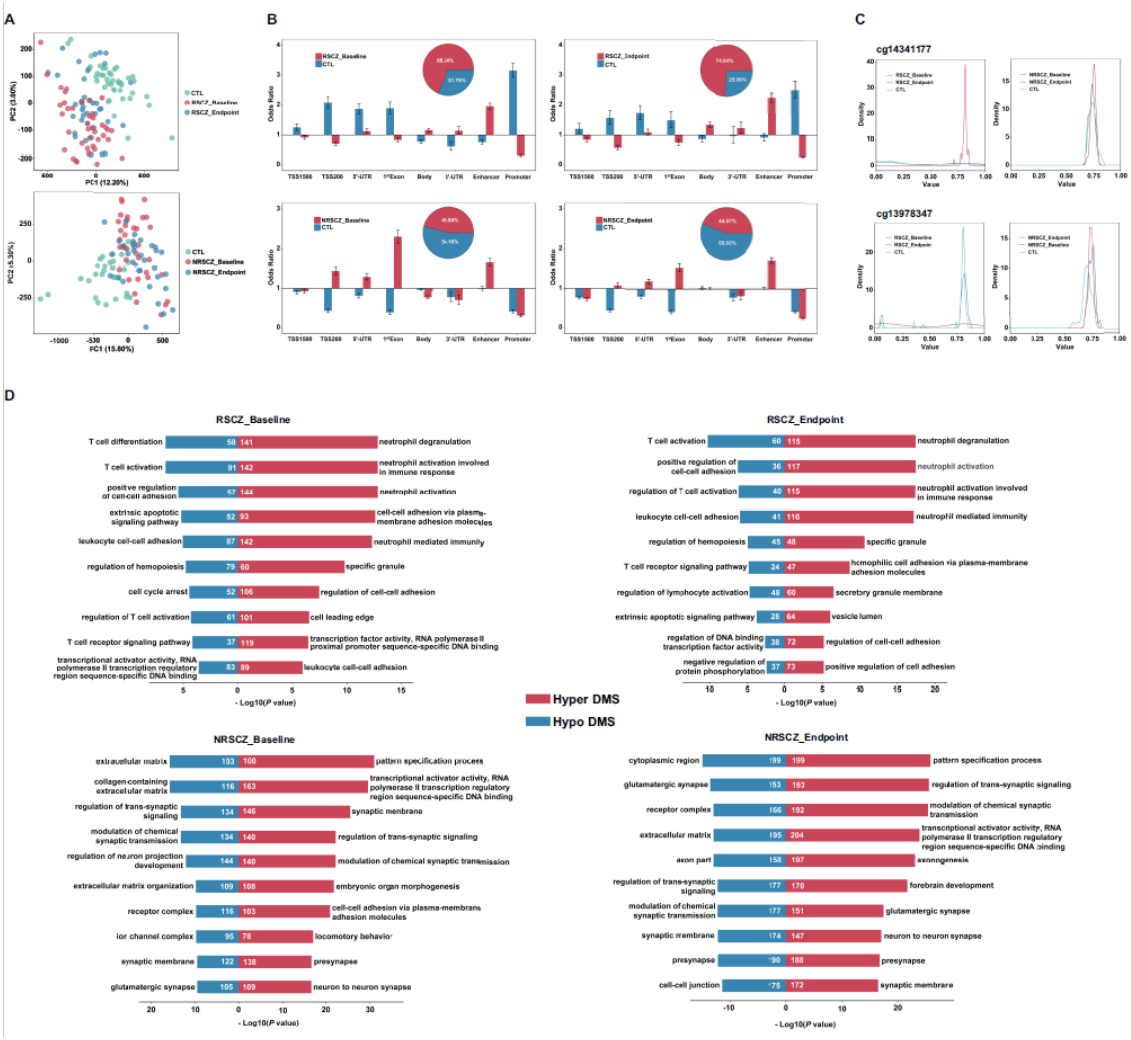


**Figure 1**

Group differences in DNA methylation profiles. (A) Principal component analysis (PCA) of DNA methylation profiles for all 120 individuals. Green, red and blue circles represent health control, baseline schizophrenia and endpoint schizophrenia, respectively. The proportions of variance explained by PC1 and PC2 are indicated. (B) Genomic location of differentially methylated sites (DMS), for CpG sites hyper-methylated in SCZ patients (red) and in controls (blue). Odds ratio and 95% confidence intervals are displayed, comparing their localization in different genomic locations as provided by Illumina and ChromHMM phase 15

(TSS1500, TSS200, 5'-UTR, 1st Exon, Body, 3'-UTR, Enhancer and promoter. Odds ratios were computed against the general distribution of the total detected CpGs of our dataset. Proportion of DMS those are hyper-methylated either in SCZ (red) or in CTL (blue) individuals. (C) Illustration of the foremost hyper-methylated DMS and hypo-methylated DMS in SCZ. The density of β values of CpG sites by category is given as illustrations of the group differences, with red, blue and green lines representing the methylation density in baseline schizophrenia, endpoint schizophrenia and control, respectively. (D) Gene Ontology (GO) enrichment analyses. For both groups, the top 10 GO categories reaching 5% FDR are shown, together with the number of genes per category and the log10-transformed FDR-adjusted enrichment P values.

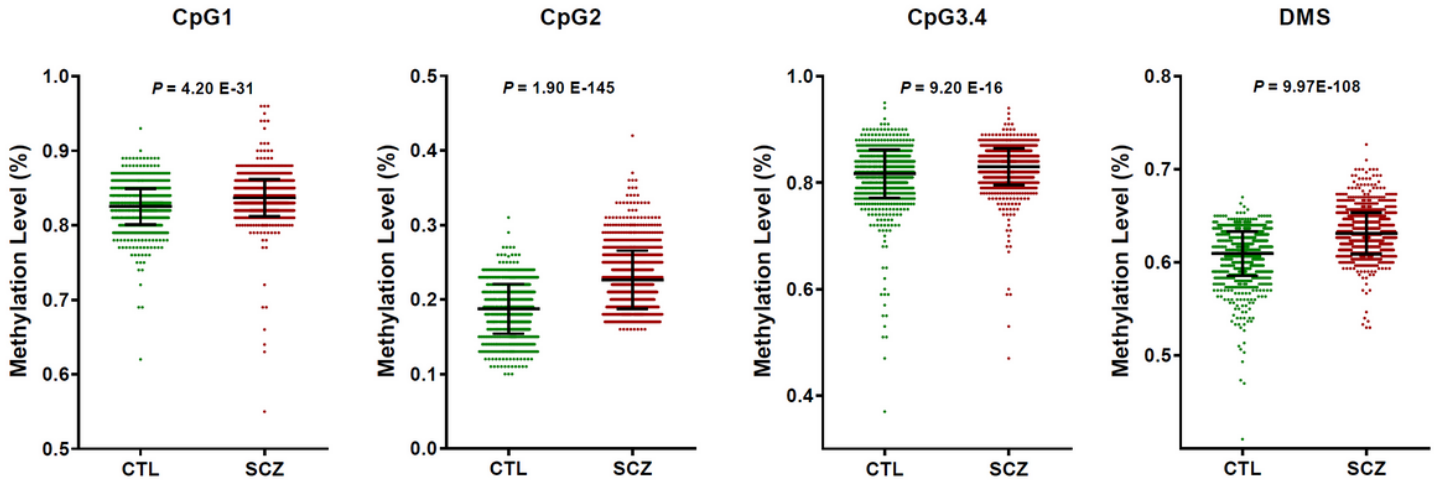


Figure 2

Quantitative methylation assay of cg14341177 in validation cohort. CTL, control; SCZ, schizophrenia; DMS, differential methylation site. Each dot represents one donor.

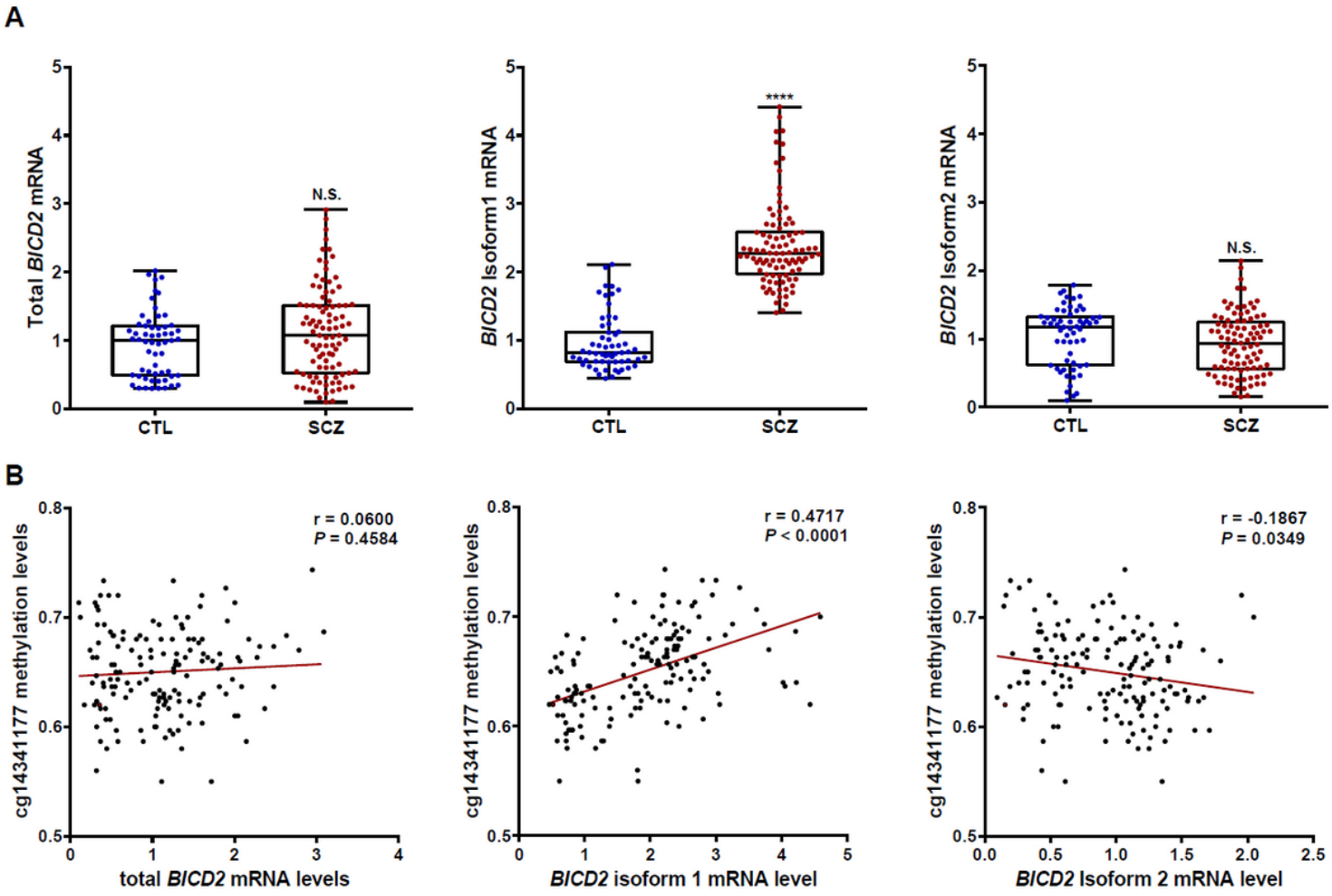


Figure 3

Hyper-methylation of cg14341177 may inhibit Exon7 alternative splicing of BICD2 mRNA. (A) Quantification of BICD2 transcripts by Taqman-probe qRT-PCR in 59 CTL and 96 SCZ (normalized to GAPDH for total BICD2 or to total BICD2 for BICD2 isoform 1 and BICD2 isoform 2). (B) Pearson correlation analysis between the level of cg14341177 methylation and the level of BICD2 transcripts. Box, 1st quartile, 3rd quartile and median; each dot represents one donor; NS, not significant; **** P < 0.0001.

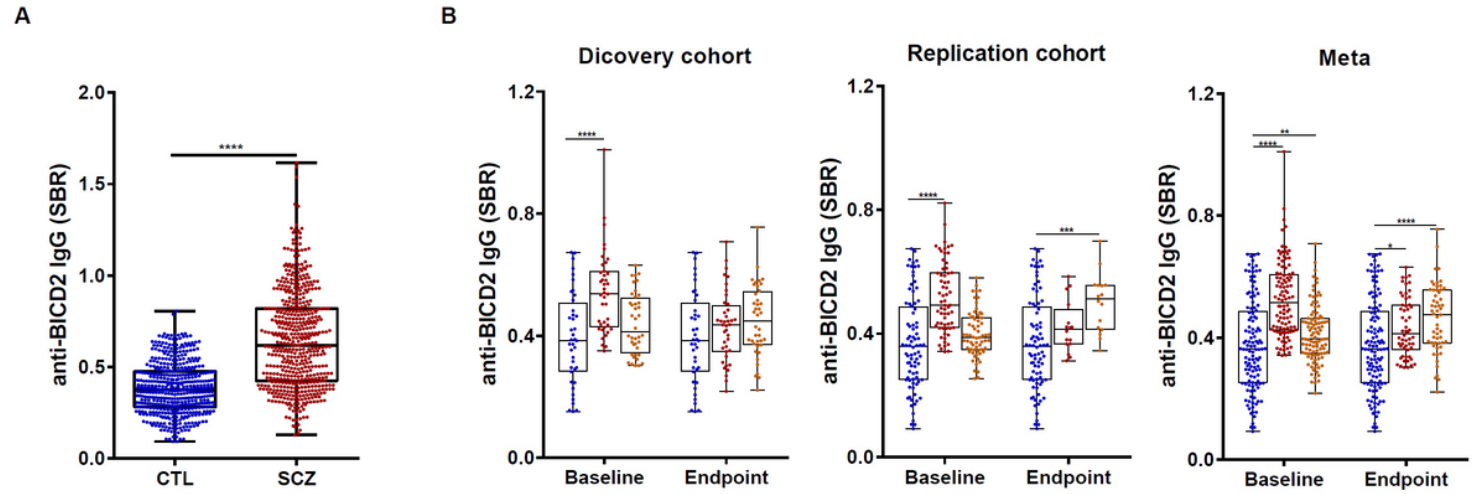


Figure 4

Upregulation of plasma anti-BICD2 IgG levels in schizophrenia. (A) Quantification of anti-BICD2 IgG levels in all available plasma samples (486 CTL v.s. 525 SCZ). (B) Comparison of the anti-BICD2 IgG changes in the discovery cohort (40 CTL, 40 RSCZ and 40 NRSCZ) and the replication cohort (84 CTL, 64 RSCZ and 16 NRSCZ). Box, 1st quartile, 3rd quartile and median; each dot represents one donor; * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

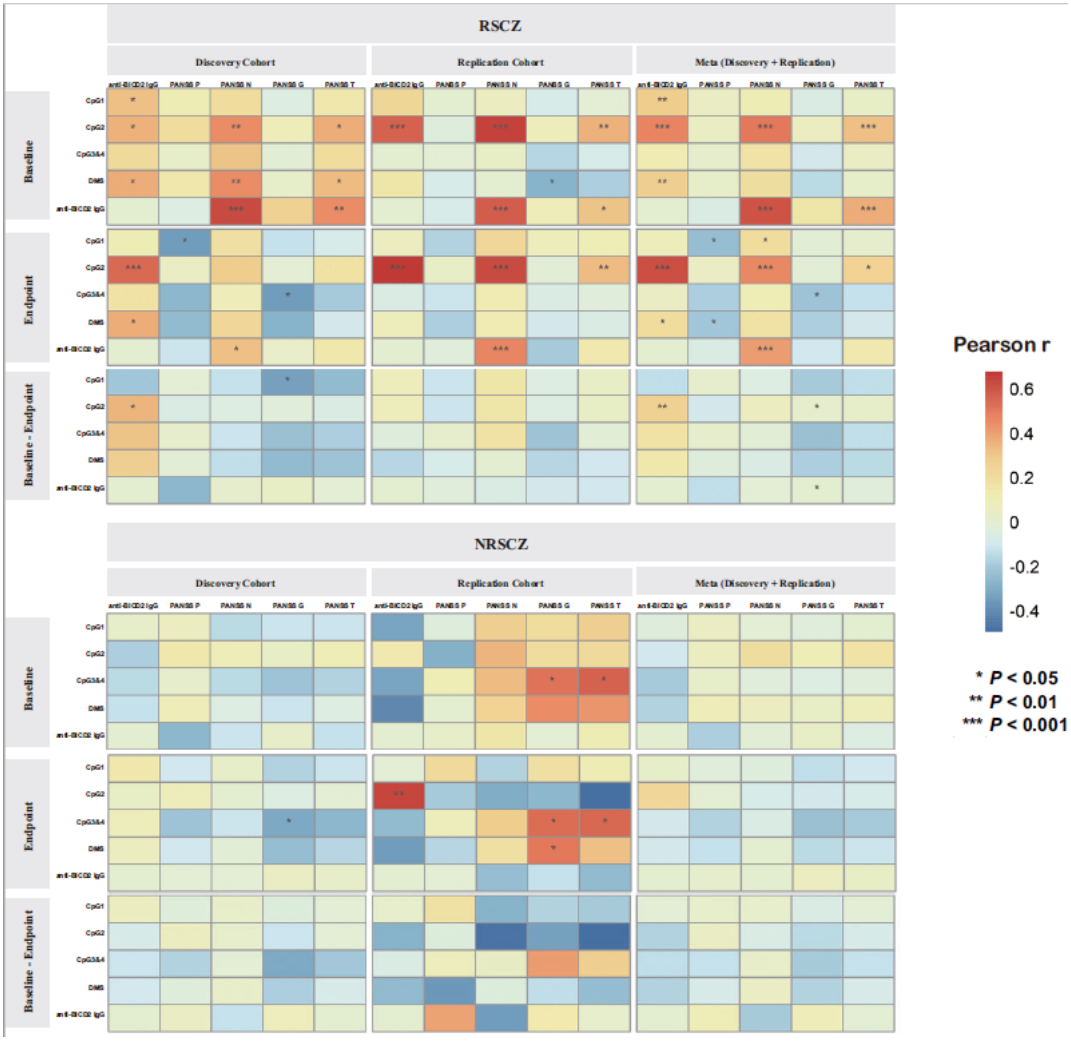


Figure 5

Correlation matrix of cg14341177 methylation, anti-BICD2 IgG and PANSS criteria scores. The darker the color, the larger the magnitude of pearson r. * P < 0.05, ** P < 0.01, *** P < 0.001.

## Supplementary Files

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