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

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# Subjects

Three hundred and eleven subjects, including 161 schizophrenia patients (82 males, 79 females) and 150 healthy controls (80 males, 70 females), were recruited in this study. All patients were recruited at the Mental Health Center of West China Hospital, Sichuan University from August 2005 to October 2012. Healthy controls were recruited from the local area through poster advertisement. Both groups were matched for sex, age and education level. All participants were right-handed based on the Annett Handedness Scale (Annett, 1970). The study was approved by the ethical committee of the West China Hospital of Sichuan University.

The patients were interviewed by an experienced psychiatrist using the Structured Clinical Interview for DSM-IV (SCID-P; First et al. 1997). DSM-IV criteria for schizophrenia or schizophreniform psychosis were used for diagnosis (APA, 1994). Patients diagnosed with schizophreniform psychosis were followed up for at least 6 months and all met the DSM-IV criteria for schizophrenia. Psychopathology associated with schizophrenia was evaluated using the positive and negative syndrome scales (PANSS), which provided the total score and five factor scores including negative, positive, excited, emotion, disorganized (Gaag et al. 2006). All controls were screened with the SCID non-patient version (SCID-NP; First et al. 1997) for the lifetime absence of psychiatric illnesses. Subjects with organic brain disorders, alcohol and/or drug abuse, pregnancy or any other severe physical illness such as brain tumor or epilepsy were excluded from the study.

# Imaging data acquisition

All participants were scanned using a 3-T MRI system (Excite, General Electric, USA). Functional MRI (fMRI) images were obtained using a gradient-echo-planar imaging (EPI) sequence (repetition time [TR]/echo time [TE], 2000/30 ms; flip angle, 90x; slice thickness, 5 mm; no slice gap; in-plane resolution, 3.75 × 3.75 mm2; matrix size, 64 × 64; field of view [FOV], 240 × 240 mm2; slice number, 30]. Each resting-state fMRI scan contained 200 image volumes. During the fMRI scan, the participants were instructed to lie still with their eyes closed, remain relaxed, and refrain from focusing on any particular thought.

## Data processing

### Imaging data processing

To remove the sources of spurious correlations present in resting-state BOLD data, all fMRI time series underwent high-pass temporal filtering (0.01 Hz), removal of nuisance signals from ventricles, deep white matter, and six rigid-body motion correction parameters, followed by low-pass temporal filtering (0.08 Hz). In addition, given the growing concerns that excessive movement can influence between-group differences, therefore, we used four procedures to achieve motion correction. In the first step, we carried out three-dimensional (3D) motion correction by aligning each functional volume to the mean image of all volumes, and any data affected by head motion of >3 mm or a rotation of > were excluded from the analysis. In the second step, we implemented additional careful volume censoring (“scrubbing”) for movement correction as reported by Power et al. to ensure that head-motion artifacts do not drive the observed effects(Power *et al.*, 2012). The mean framewise displacement (FD) was computed with an FD threshold for displacement of 0.5. In addition to the frame corresponding to the displaced time point, one preceding and two succeeding time points were also deleted to reduce the “spill-over” effect of head movements. Third, subjects with >10% displaced frames flagged were completely excluded from the analysis, as such high level of movement is likely to have influenced several volumes. Finally, the mean displacements were computed as root mean square of the translation and rotation parameters (computed as the average of the absolute value of the Euler angle of the rotation of each brain volume compared to the previous volume). We used the root mean square displacement as a covariate when comparing the two groups during statistical analysis.

## Genotyping and quality controls

DNA was extracted from whole blood samples using the standard isolation method. Genotyping was performed on the HumanOmniZhongHua-8 BeadChip (Illumina, San Diego, CA). Genotyping data were systematically filtered according to their genotyping rates, minor allele frequency (MAF), and Hardy-Weinberg equilibrium tests (only in controls). Participants with low genotyping rate (<97%), markers with >5% missing rate per individual and/or with MAF > 0.05, and markers that failed to pass the Hardy-Weinberg equilibrium tests (p ≤ 10–6) were excluded from further analysis. Furthermore, the gender of each participant, with genotyping data on gender-specific loci, was confirmed by the genotyping platform, and participants with lower genotype call rates were excluded from the analysis if participant pairs with identical genotypes were found. After systematic filtering, 1,09,923 SNPs and four individuals (two patients and two healthy controls) were excluded from the study based on the above criteria. All quality control analyses were performed using PLINK(Purcell *et al.*, 2007). The standard pipeline was used to detect outliers, and population stratification was carried out using EIGENSTART.(Price *et al.*, 2006) The first two components of the PCA results were included as covariates in the subsequent association test.

Table S Significant regions in the voxel-based whole brain analysis.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **No.** | **Areas** | **Cluster size**  **#Voxels** | **Peak MA value** | **MNI coordinates (Peak)** |
| **Cluster 1** | Rectus\_L, Frontal\_Sup\_Orb\_L | 45 | 92 | -12 48 -18 |
| **Cluster 2** | Thalamus\_L | 69 | 33 | -12 -12 6 |
| **Cluster 3** | Thalamus\_R | 50 | 21 | 12 -12 6 |

Table S GWAS results of top significant interaction (SNPs x diagnosis) of 4 functional connectivities using LMM

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Trait** | **CHR** | **SNP** | **BP** | **ß** | **P** | **SNPType** | **Nearest Genes(2k)** |
| REC.L\_THA.R | 1 | rs6700381 | 239519108 | 0.284291401 | 1.76802E-08 | genotyped | *CHRM3* |
| REC.L\_THA.L | 1 | rs6700381 | 239519108 | 0.285472805 | 2.7211E-08 | genotyped | *CHRM3* |
| REC.L\_THA.R | 14 | rs970014 | 21222926 | 0.034867879 | 1.43218E-07 | genotyped | *FAM12A* |
| REC.L\_THA.R | 14 | rs970015 | 21222991 | 0.034867879 | 1.43218E-07 | genotyped | *FAM12A* |
| REC.L\_THA.R | 1 | rs10925873 | 239515374 | 0.270743258 | 1.88038E-07 | imputed | *CHRM3* |
| REC.L\_THA.R | 14 | rs12886881 | 21227181 | 0.038104375 | 2.08755E-07 | imputed | *FAM12A* |
| REC.L\_THA.R | 14 | rs10131121 | 21231575 | 0.038104375 | 2.08755E-07 | imputed | *FAM12A* |
| REC.L\_THA.L | 1 | rs10925873 | 239515374 | 0.274818027 | 2.20459E-07 | imputed | *CHRM3* |
| REC.L\_THA.R | 14 | rs2073343 | 21230530 | 0.037192459 | 2.31507E-07 | imputed | *FAM12A* |
| REC.L\_THA.L | 14 | rs970014 | 21222926 | 0.057383566 | 2.90297E-07 | genotyped | *FAM12A* |
| REC.L\_THA.L | 14 | rs970015 | 21222991 | 0.057383566 | 2.90297E-07 | genotyped | *FAM12A* |
| REC.L\_THA.L | 14 | rs12886881 | 21227181 | 0.063068255 | 3.03992E-07 | imputed | *FAM12A* |
| REC.L\_THA.L | 14 | rs10131121 | 21231575 | 0.063068255 | 3.03992E-07 | imputed | *FAM12A* |
| REC.L\_THA.L | 14 | rs2073343 | 21230530 | 0.061855731 | 3.15751E-07 | imputed | *FAM12A* |
| ORBsup.L\_THA.R | 15 | rs4778338 | 24089570 | 0.400215192 | 5.62538E-07 | imputed | *NDN* |
| ORBsup.L\_THA.R | 15 | rs17813715 | 24089841 | 0.400215192 | 5.62538E-07 | imputed | *NDN* |
| ORBsup.L\_THA.R | 3 | rs10510929 | 64734036 | -1.65849E-05 | 5.92998E-07 | imputed | *MAGI1* |
| ORBsup.L\_THA.L | 5 | rs2541312 | 82819185 | 0.357347032 | 6.18444E-07 | genotyped | *XRCC4* |
| ORBsup.L\_THA.R | 5 | rs644685 | 18066953 | -0.260297783 | 6.33189E-07 | imputed | *LOC646241* |
| ORBsup.L\_THA.R | 15 | rs35037564 | 24094041 | 0.396905153 | 6.49534E-07 | imputed | *NDN* |
| REC.L\_THA.R | 1 | rs10158639 | 239507300 | 0.266653336 | 6.84596E-07 | imputed | *CHRM3* |
| ORBsup.L\_THA.R | 15 | rs7173902 | 24088014 | 0.396116607 | 7.30939E-07 | imputed | *NDN* |
| ORBsup.L\_THA.R | 15 | rs8039055 | 24088507 | 0.396116607 | 7.30939E-07 | imputed | *NDN* |
| ORBsup.L\_THA.R | 15 | rs12438172 | 24088975 | 0.396116607 | 7.30939E-07 | imputed | *NDN* |
| ORBsup.L\_THA.R | 15 | rs12438220 | 24089140 | 0.396116607 | 7.30939E-07 | imputed | *NDN* |
| ORBsup.L\_THA.R | 15 | rs1120970 | 24089418 | 0.396116607 | 7.30939E-07 | imputed | *NDN* |
| REC.L\_THA.L | 14 | rs61976922 | 21228552 | 0.055205849 | 7.46137E-07 | imputed | *FAM12A* |
| ORBsup.L\_THA.R | 15 | rs4778336 | 24084757 | 0.402900646 | 7.86501E-07 | imputed | *NDN* |
| ORBsup.L\_THA.L | 1 | rs6700381 | 239519108 | 0.254473276 | 8.06227E-07 | genotyped | *CHRM3* |
| REC.L\_THA.R | 15 | rs4778338 | 24089570 | 0.37339048 | 8.17569E-07 | imputed | *NDN* |
| REC.L\_THA.R | 15 | rs17813715 | 24089841 | 0.37339048 | 8.17569E-07 | imputed | *NDN* |
| ORBsup.L\_THA.R | 14 | rs970014 | 21222926 | 0.013820692 | 8.21771E-07 | genotyped | *FAM12A* |
| ORBsup.L\_THA.R | 14 | rs970015 | 21222991 | 0.013820692 | 8.21771E-07 | genotyped | *FAM12A* |
| REC.L\_THA.L | 1 | rs10158639 | 239507300 | 0.270178628 | 8.24164E-07 | imputed | *CHRM3* |
| REC.L\_THA.R | 15 | rs35037564 | 24094041 | 0.369778403 | 9.0214E-07 | imputed | *NDN* |
| ORBsup.L\_THA.R | 15 | rs17757180 | 24082747 | 0.398621058 | 9.14497E-07 | genotyped | *NDN* |
| ORBsup.L\_THA.R | 15 | rs6576643 | 24082586 | 0.398621058 | 9.14497E-07 | imputed | *NDN* |
| ORBsup.L\_THA.L | 8 | rs2921048 | 8320834 | 0.090284692 | 9.18676E-07 | imputed | *PRAGMIN* |
| ORBsup.L\_THA.L | 8 | rs2980770 | 8320938 | 0.090284692 | 9.18676E-07 | imputed | *PRAGMIN* |
| REC.L\_THA.R | 15 | rs7173902 | 24088014 | 0.368958481 | 9.52346E-07 | imputed | *NDN* |
| REC.L\_THA.R | 15 | rs8039055 | 24088507 | 0.368958481 | 9.52346E-07 | imputed | *NDN* |
| REC.L\_THA.R | 15 | rs12438172 | 24088975 | 0.368958481 | 9.52346E-07 | imputed | *NDN* |
| REC.L\_THA.R | 15 | rs12438220 | 24089140 | 0.368958481 | 9.52346E-07 | imputed | *NDN* |
| REC.L\_THA.R | 15 | rs1120970 | 24089418 | 0.368958481 | 9.52346E-07 | imputed | *NDN* |
| REC.L\_THA.R | 3 | rs55964533 | 141007602 | 0.003508191 | 9.74855E-07 | imputed | *SPSB4* |
| REC.L\_THA.R | 3 | rs56357034 | 141007644 | 0.003508191 | 9.74855E-07 | imputed | *SPSB4* |
| REC.L\_THA.R | 5 | rs249496 | 102201590 | -0.109453165 | 1.02522E-06 | genotyped | *SLCO6A1* |
| REC.L\_THA.R | 15 | rs34440064 | 24086224 | 0.347687206 | 1.36796E-06 | imputed | *NDN* |
| REC.L\_THA.R | 7 | rs17144737 | 21631483 | 0.485124845 | 1.41211E-06 | imputed | *SP4* |
| ORBsup.L\_THA.R | 8 | rs13264265 | 2095689 | 0.036292518 | 1.47865E-06 | imputed | *MYOM2* |
| ORBsup.L\_THA.R | 1 | rs6700381 | 239519108 | 0.246835195 | 1.49915E-06 | genotyped | *CHRM3* |
| ORBsup.L\_THA.R | 14 | rs2073343 | 21230530 | 0.010504713 | 1.52707E-06 | imputed | *FAM12A* |
| ORBsup.L\_THA.R | 8 | rs1520930 | 64700894 | 0.153726933 | 1.56925E-06 | imputed | *IFITM8P* |
| ORBsup.L\_THA.R | 14 | rs12886881 | 21227181 | 0.013290404 | 1.68489E-06 | imputed | *FAM12A* |
| ORBsup.L\_THA.R | 14 | rs10131121 | 21231575 | 0.013290404 | 1.68489E-06 | imputed | *FAM12A* |
| ORBsup.L\_THA.R | 5 | rs249496 | 102201590 | -0.07461314 | 1.69045E-06 | genotyped | *SLCO6A1* |
| REC.L\_THA.L | 10 | rs242972 | 119225250 | -0.00925799 | 1.69143E-06 | imputed | *PDZD8* |
| ORBsup.L\_THA.R | 1 | rs443061 | 6810718 | 0.616273331 | 1.90901E-06 | imputed | *DNAJC11* |
| ORBsup.L\_THA.L | 8 | rs2921044 | 8325753 | 0.080772375 | 2.00573E-06 | genotyped | *PRAGMIN* |
| ORBsup.L\_THA.L | 8 | rs2921045 | 8325744 | 0.080772375 | 2.00573E-06 | imputed | *PRAGMIN* |
| REC.L\_THA.R | 3 | rs16851316 | 141006582 | -0.03202482 | 2.19835E-06 | imputed | *SPSB4* |
| ORBsup.L\_THA.L | 1 | rs10925873 | 239515374 | 0.246491066 | 2.21804E-06 | imputed | *CHRM3* |
| REC.L\_THA.R | 15 | rs4778336 | 24084757 | 0.367922707 | 2.33038E-06 | imputed | *NDN* |
| REC.L\_THA.R | 3 | rs6779015 | 141007003 | -0.022223825 | 2.35361E-06 | genotyped | *SPSB4* |
| REC.L\_THA.L | 8 | rs2921048 | 8320834 | 0.081128554 | 2.4008E-06 | imputed | *PRAGMIN* |
| REC.L\_THA.L | 8 | rs2980770 | 8320938 | 0.081128554 | 2.4008E-06 | imputed | *PRAGMIN* |
| ORBsup.L\_THA.R | 15 | rs34440064 | 24086224 | 0.36212592 | 2.44197E-06 | imputed | *NDN* |
| ORBsup.L\_THA.L | 8 | rs2979182 | 8325448 | 0.081375547 | 2.45955E-06 | imputed | *PRAGMIN* |
| REC.L\_THA.R | 15 | rs17757180 | 24082747 | 0.364462739 | 2.51157E-06 | genotyped | *NDN* |
| REC.L\_THA.R | 15 | rs6576643 | 24082586 | 0.364462739 | 2.51157E-06 | imputed | *NDN* |
| REC.L\_THA.R | 14 | rs2145870 | 21218664 | 9.37541E-05 | 2.59448E-06 | imputed | *FAM12A* |
| REC.L\_THA.R | 14 | rs2145871 | 21218700 | 9.37541E-05 | 2.59448E-06 | imputed | *FAM12A* |
| ORBsup.L\_THA.R | 1 | rs10925873 | 239515374 | 0.24057791 | 2.62095E-06 | imputed | *CHRM3* |
| ORBsup.L\_THA.R | 3 | rs10937420 | 189795575 | 0.224104876 | 2.65706E-06 | genotyped | *TP63* |
| ORBsup.L\_THA.R | 3 | rs4334626 | 189790012 | 0.224104876 | 2.65706E-06 | imputed | *TP63* |
| ORBsup.L\_THA.R | 3 | rs35423000 | 189790480 | 0.224104876 | 2.65706E-06 | imputed | *TP63* |
| ORBsup.L\_THA.R | 3 | rs9836307 | 189790557 | 0.224104876 | 2.65706E-06 | imputed | *TP63* |
| ORBsup.L\_THA.R | 3 | rs9873955 | 189790606 | 0.224104876 | 2.65706E-06 | imputed | *TP63* |
| ORBsup.L\_THA.R | 3 | rs1403414 | 189791147 | 0.224104876 | 2.65706E-06 | imputed | *TP63* |
| ORBsup.L\_THA.R | 3 | rs6444415 | 189791727 | 0.224104876 | 2.65706E-06 | imputed | *TP63* |
| ORBsup.L\_THA.R | 3 | rs6781725 | 189792069 | 0.224104876 | 2.65706E-06 | imputed | *TP63* |
| REC.L\_THA.R | 6 | rs2743021 | 104509741 | 0.119602333 | 2.68577E-06 | imputed | *HACE1* |
| ORBsup.L\_THA.L | 3 | rs10510929 | 64734036 | -0.009493685 | 2.71903E-06 | imputed | *MAGI1* |
| ORBsup.L\_THA.L | 1 | rs10158639 | 239507300 | 0.248174063 | 2.75059E-06 | imputed | *CHRM3* |
| REC.L\_THA.R | 8 | rs1520930 | 64700894 | 0.140367048 | 2.79227E-06 | imputed | *IFITM8P* |
| ORBsup.L\_THA.R | 20 | rs6091231 | 49494019 | 0.308823165 | 2.87335E-06 | genotyped | *ADNP* |
| ORBsup.L\_THA.R | 1 | rs10158639 | 239507300 | 0.242374115 | 3.27937E-06 | imputed | *CHRM3* |
| REC.L\_THA.R | 13 | rs3903948 | 80619400 | 0.380967111 | 3.35011E-06 | genotyped | *LOC729485* |
| REC.L\_THA.L | 14 | rs2142187 | 78057835 | -0.627231472 | 3.87943E-06 | imputed | *ALKBH1* |
| REC.L\_THA.R | 14 | rs61976922 | 21228552 | 0.019372949 | 3.92E-06 | imputed | *FAM12A* |
| REC.L\_THA.L | 1 | rs3842542 | 82316524 | -0.256594027 | 4.19544E-06 | imputed | *TTLL7* |
| REC.L\_THA.L | 8 | rs2921044 | 8325753 | 0.074226312 | 4.54624E-06 | genotyped | *PRAGMIN* |
| REC.L\_THA.L | 8 | rs2921045 | 8325744 | 0.074226312 | 4.54624E-06 | imputed | *PRAGMIN* |
| ORBsup.L\_THA.L | 14 | rs970014 | 21222926 | 0.047380093 | 4.67913E-06 | genotyped | *FAM12A* |
| ORBsup.L\_THA.L | 14 | rs970015 | 21222991 | 0.047380093 | 4.67913E-06 | genotyped | *FAM12A* |
| ORBsup.L\_THA.L | 20 | rs41308639 | 2473587 | -0.513125749 | 4.73571E-06 | imputed | *TMC2* |
| REC.L\_THA.L | 6 | rs7767963 | 66245983 | -0.007756585 | 4.91813E-06 | genotyped | *RLOC442229* |
| REC.L\_THA.L | 6 | rs12525235 | 66236140 | -0.007756585 | 4.91813E-06 | imputed | *RLOC442229* |
| REC.L\_THA.L | 6 | rs12530329 | 66240459 | -0.007756585 | 4.91813E-06 | imputed | *RLOC442229* |
| REC.L\_THA.L | 6 | rs16896773 | 66241831 | -0.007756585 | 4.91813E-06 | imputed | *RLOC442229* |
| REC.L\_THA.L | 6 | rs57672364 | 66244363 | -0.007756585 | 4.91813E-06 | imputed | *RLOC442229* |
| REC.L\_THA.L | 6 | rs10080847 | 66247059 | -0.007756585 | 4.91813E-06 | imputed | *RLOC442229* |
| TATREC.L\_THA.RS | 1 | rs6700381 | 239519108 |  | 5.13E-08 | genotyped | *CHRM3* |
| TATREC.L\_THA.RS | 14 | rs970014 | 21222926 |  | 4.14E-07 | genotyped | *FAM12A* |
| TATREC.L\_THA.RS | 14 | rs970015 | 21222991 |  | 4.14E-07 | genotyped | *FAM12A* |
| TATREC.L\_THA.RS | 1 | rs10925873 | 239515374 |  | 4.87E-07 | imputed | *CHRM3* |
| TATREC.L\_THA.RS | 14 | rs12886881 | 21227181 |  | 6.03E-07 | imputed | *FAM12A* |
| TATREC.L\_THA.RS | 14 | rs10131121 | 21231575 |  | 6.03E-07 | imputed | *FAM12A* |
| TATREC.L\_THA.RS | 14 | rs2073343 | 21230530 |  | 6.69E-07 | imputed | *FAM12A* |

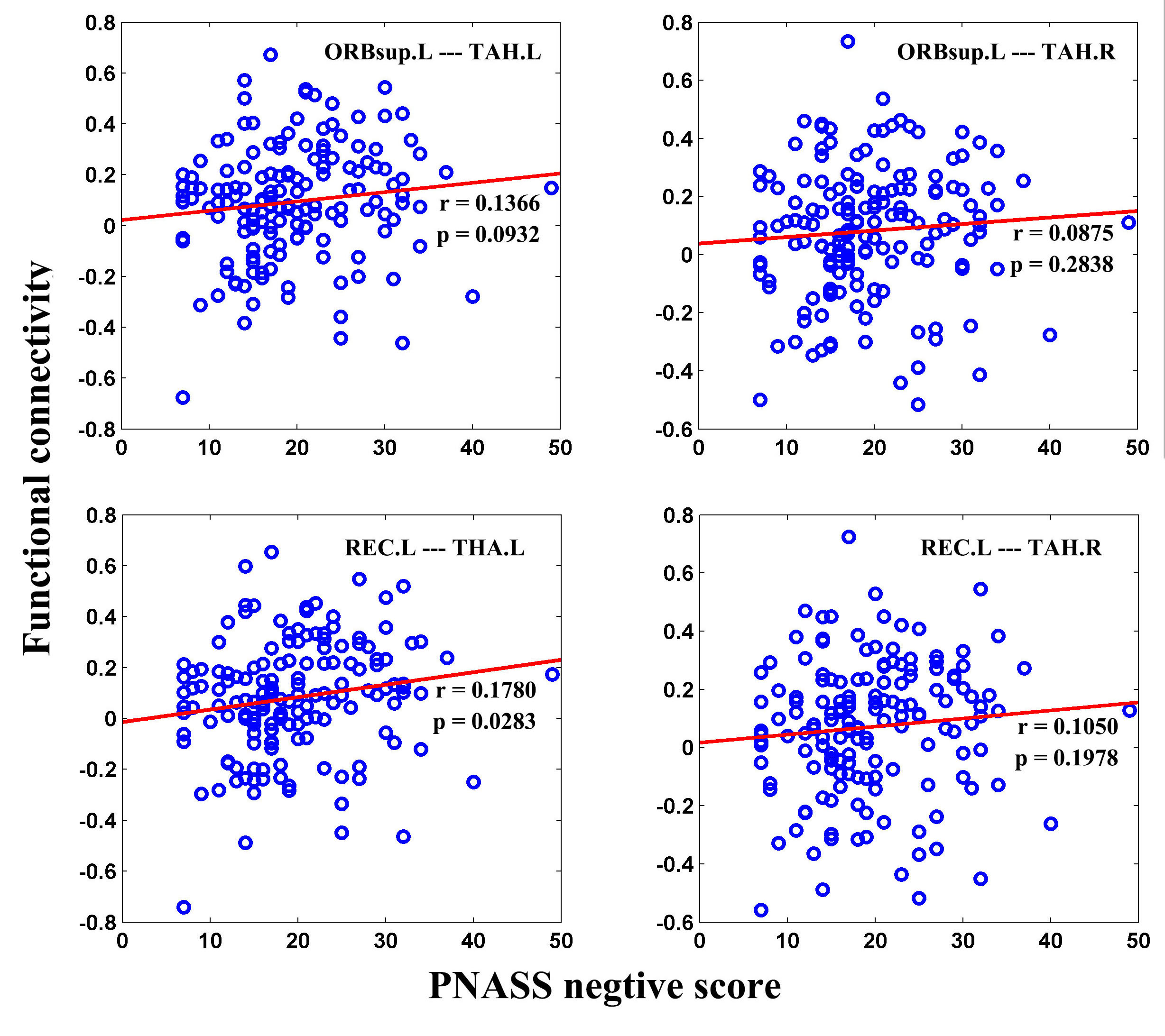


Figure S the correlation of negative symptoms with altered functional connectivities using the cluster-based ROI-wise functional connectivity measures identified. There is a weak correlation between negative symptoms and the connectivities of REC.L-THA.L, but the correlation could not survive after multiple corrections. There are no any correlation amongst other clinical manifestations and altered functional connectivities.

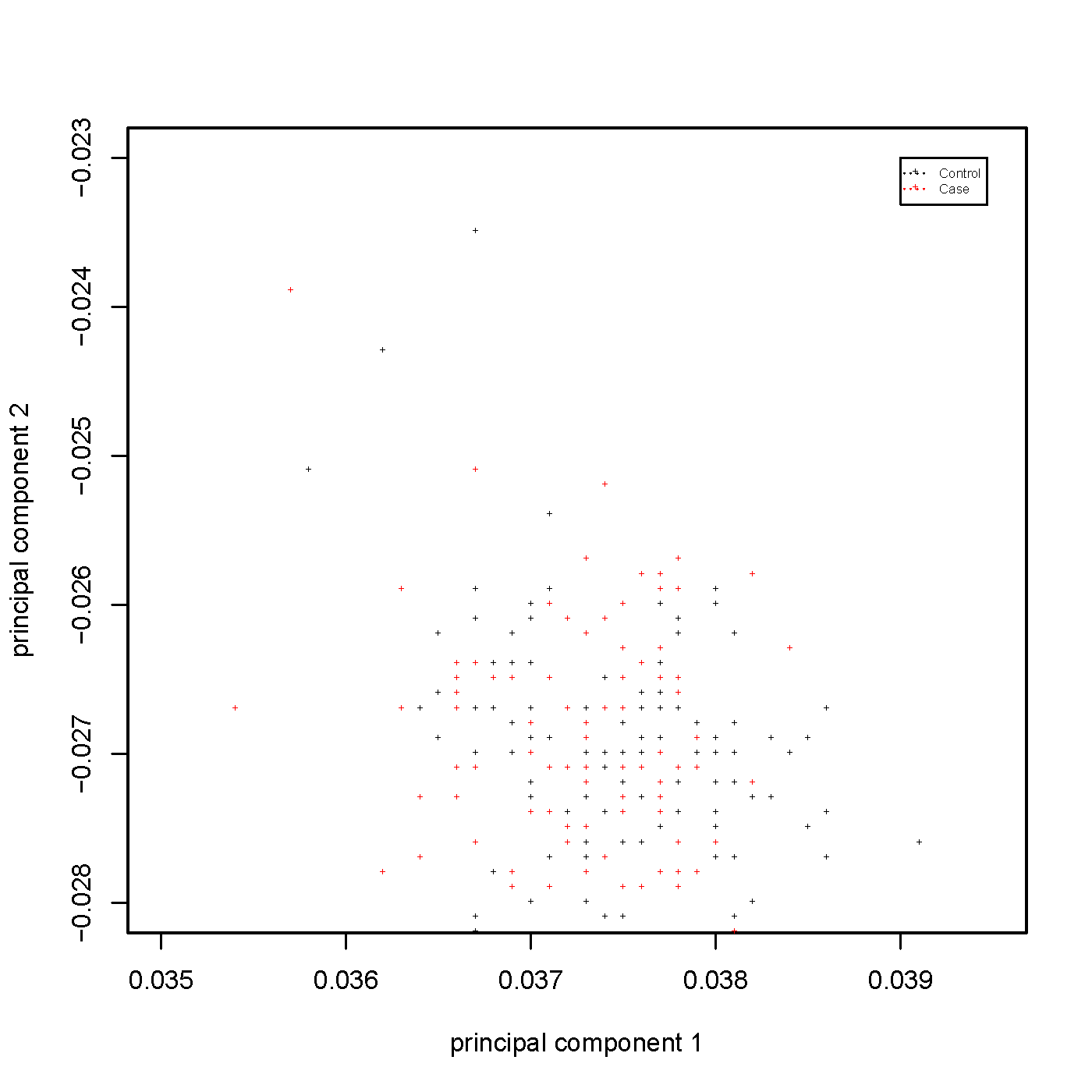


Figure S The plot of the first two principal components of 127 patients with schizophrenia and 100 controls as output from EIGENSOFT(Price *et al.*, 2006). Each data point represents an individual.



Figure S QQ plot of linear mixed model of 4 univariate analysis.

## References

**Power JD, Barnes KA, Snyder AZ, Schlaggar BL & Petersen SE** (2012). Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *Neuroimage* **59**, 2142-2154.

**Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA & Reich D** (2006). Principal components analysis corrects for stratification in genome-wide association studies. *Nature Genetics* **38**, 904-909.

**Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, De Bakker PIW & Daly MJ** (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *The American Journal of Human Genetics* **81**, 559-575.