Genetic association analysis of microRNA137 and its target complex 1 with schizophrenia in Han Chinese

Weihong Lu1, Yi Zhang1, Xinyu Fang1, Weixing Fan2, Wei Tang3, Jun Cai1, Lisheng Song1 & Chen Zhang1

Recent genome-wide association studies (GWAS) have identified a strong association signal of microRNA137 host gene (MIR137) with schizophrenia. MIR137 dysfunction results in downregulation of presynaptic target gene complexin 1 (CPLX1) and impairs synaptic plasticity in the hippocampus. In this study, we aimed to investigate whether the variants of MIR137 and CPLX1 confer susceptibility to schizophrenia in Han Chinese. This study employed 736 patients with schizophrenia patients and 751 well-matched healthy subjects for genetic analysis, and genotyped 12 SNPs within MIR137 and CPLX1. SZDB database was used to performed brain eQTL analysis. There were no significant differences of CPLX1 expression in hippocampus, prefrontal cortex or stratum between the schizophrenia patients and control subjects. No significant differences were observed in allele and genotype frequencies in studied SNPs between the case and control groups. Gene interaction analysis showed that MIR137 SNP rs1625579 did not affect schizophrenia susceptibility in interaction with the CPLX1 polymorphic variants. Our findings do not support MIR137 and CPLX1 conferring susceptibility to schizophrenia in Han Chinese.

Schizophrenia is a severe and disabling mental illness with clinical symptoms typically manifesting in a late adolescence or early adulthood onset. Although its etiology and pathophysiology remain unknown, the underlying cause of schizophrenia is suspected to a disruption of early brain development resulted from genetic predisposition and prenatal/perinatal environment factors1. A variety of genetic risks identified in schizophrenia are genes expressing proteins involved in the regulation of synaptic plasticity2.

Recent genome-wide association studies (GWAS) have identified a strong association signal of microRNA137 host gene (MIR137) with schizophrenia3–5. MicroRNAs (miRNAs) are small noncoding single-stranded RNAs that function as post-transcriptional regulators of gene expression6. In the central nervous system, miRNAs may play an important role in neurodevelopment and maturation including synaptic development, dendritic protein synthesis and neural plasticity7. MicroRNA137 is a brain-enriched miRNA in human with high expression in cortical brain regions and hippocampus, and has a critical regulatory role in brain function8–10. At the molecular level, a single nucleotide polymorphism (SNP) rs1625579 in MIR137 has been reported to confer susceptibility to schizophrenia in populations of European ancestry. However, the association of rs1625579 with schizophrenia is inconsistent in Asian populations11–17. Pu and Xiao18 thereby performed a meta-analysis and provided unsupportive evidence for the association of rs1625579 with schizophrenia in Asians.

A recent study has pointed out that MIR137 dysfunction results in downregulation of presynaptic target gene complexin-1 (CPLX1) and impairs synaptic plasticity in the hippocampus in vitro and in vivo19. Complexin has a regulatory role in synaptic vesicle exocytosis20 and complexin 1 modulates vesicle release21. A postmortem study reported that patients with schizophrenia have a significant decrease of CPLX1 protein in prefrontal cortex, when compared with healthy subjects22. However, an early genetic study scanned the haplotype-tagging23 SNPs in CPLX1 in a small sample of Japanese patients with schizophrenia, whereas no significant association of CPLX1

1Schizophrenia Program, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China. 2Department of Psychiatry, Jinhua Second Hospital, Jinhua, Zhejiang, China. 3Department of Psychiatry, Wenzhou Kangning Hospital, Wenzhou, Zhejiang, China. Correspondence and requests for materials should be addressed to C.Z. (email: zhangchen645@gmail.com)
with schizophrenia was observed\(^2\). As abovementioned, CPLX1 is downregulated by miRNA137 gain of function, causing impairment in synaptic vesicle trafficking and alterations in synaptic plasticity\(^1\). Therefore, we hypothesized that the potential interaction effect of MIR137 and CPLX1 may influence the genetic risk for schizophrenia.

In this study, we aimed to investigate whether the variants of MIR137 and CPLX1 confer susceptibility to schizophrenia in Han Chinese. Here, we first used a public database to detect whether CPLX1 differentially expressed in brain between patients with schizophrenia and healthy controls. Second, we totally genotyped twelve SNPs of MIR137 and CPLX1 in our samples. Meanwhile, we also detected the effect of the two genes interaction in the susceptibility of schizophrenia, because a specific individual genetic variant has a minor marginal effect in such a complex psychiatric disease and gene-gene interaction has importance to describe such effect\(^2\).

Results

We extracted brain CPLX1 expression data between schizophrenia patients and healthy controls from SZDB database\(^2\). Figure 1 showed that there were no significant differences of CPLX1 expression in hippocampus, prefrontal cortex or stratum between the schizophrenia patients and control subjects (corrected \(P = 0.26, 0.64, 0.84\), respectively).

None of the genotypic distributions showed deviation from the Hardy-Weinberg equilibrium. There were no significant differences in allele and genotype frequencies in any SNP of either MIR137 or CPLX1 between the case and control groups (Table 1). After calculating LD for all pairs of SNP markers in CPLX1, we found two strong LDs between rs11248043 and rs7376690, as well as rs6832751 and rs10155482 (Supplementary Figure S1). Supplementary Table S3 listed all \(P\) values corresponding to haplotypes, with rare haplotypes (\(<3\%\)) being dropped. The haplotypes showed no significant association with schizophrenia. Next, we extracted the schizophrenia genetic association data from the PGC database and observed no significant association of CPLX1 with schizophrenia either (Supplementary Figure S2). As shown in Table 2, we did not find the MIR137 SNP rs1625579 affecting schizophrenia susceptibility in interaction with the CPLX1 polymorphic variants (\(P > 0.05\)).

On the basis of the genotype data, the statistical power of all SNPs was more than 80\% (\(\alpha = 0.05\)) for our samples under the assumption of a modest effect size (OR = 1.5) and a log additive model and the disease prevalence of 1\%.

Discussion

In this study, our results did not support the involvement of MIR137 and CPLX1 in the pathophysiology of schizophrenia, at least in Han Chinese population. Although a recent meta-analysis showed that MIR137 SNP rs1625579 significantly increases the risk of schizophrenia\(^2\), another meta-analysis indicated that the association of rs1625579 with schizophrenia did not exhibit in Asian ancestry resulted from potential genetic heterogeneity between European and Asian populations\(^1\). Our results provided further evidence to support this conclusion. On the other hand, we failed to find any positive association signals between CPLX1 and schizophrenia in Han Chinese. This is in line with early literature that Kishi et al.\(^2\) scanned CPLX1 in Japanese population and observed negative association between CPLX1 and schizophrenia. Given the modulatory effect of microRNA137 on complexin 1\(^1\), we hypothesized a gene interaction between the MIR137 and CPLX1 may confer susceptibility to schizophrenia. However, our explorative analysis did not support this hypothesis.
A recent postmortem study found that transcript level for CPLX1 is significantly decreased in the anterior cingulate cortex (ACC) of schizophrenia patients\(^3\), whereas our results showed CPLX1 expression does not alter in hippocampus, prefrontal cortex or striatum of schizophrenia patients. It is generally accepted that ACC contributes to cognitive control, decision-making, empathy and emotion\(^{29,30}\). Animal experiment showed that CPLX1 knockout mice have pronounced deficits in social behaviors\(^31\). It is known that schizophrenia is characterized by persistent cognitive deficits, positive and negative symptoms and its etiological heterogeneity is manifested\(^{32–35}\). Therefore, although no association of CPLX1 with schizophrenia susceptibility was observed in our samples, we could not fully exclude the possible involvement of CPLX1 in the development of cognitive dysfunction in schizophrenia.

MicroRNA137 is enriched in hippocampal and cortical neurons that play important roles in neuronal maturation and dendritic spine morphogenesis\(^36\). It is known or predicted to regulate hundreds of genes, whose targets include many schizophrenia susceptibility genes, such as BDNF, ZNF804A, TCF4 and CACNA1C\(^{37,38}\). Therefore, MIR137 associated risk for schizophrenia may be implicated with its downstream genetic effects\(^{38}\) and any potential association with schizophrenia.

### Table 1. Comparison of allele and genotype frequencies of the selected SNPs within MIR137 and CPLX1 between schizophrenia and healthy control groups.

| SNP ID | Allele | Genotype | Number of samples | Case | Control | P-value | P-value | P-value | Allele | Number of samples | Case | Control | P-value | P-value | P-value |
|--------|--------|----------|------------------|------|---------|---------|---------|---------|--------|------------------|------|---------|---------|---------|---------|
| rs1625579 | G/T | GG/GT/TT | 94/1138 | 85/1417 | 0.40 | 0.10 | 0.59 | 1.00 | 0.37 | 1.00 | 0.49 | 1.00 | 0.31 | 1.00 |
| rs2242237 | C/T | CC/CT/TT | 38/263/435 | 46/252/453 | 0.55 | 0.24 | 0.001 | 0.002 | 0.0007 | 0.00006 | 0.00007 | 0.00003 | 0.00004 | 0.00002 |
| rs2306251 | G/A | AA/AG/GG | 56/292/388 | 65/282/404 | 0.60 | 0.97 | 0.001 | 0.003 | 0.001 | 0.00006 | 0.00002 | 0.00004 | 0.00008 | 0.00009 |
| rs1172977 | A/C | AA/AC/GC | 185/355/196 | 177/396/178 | 0.21 | 0.68 | 0.69 | 1.00 | 0.97 | 1.00 | 0.97 | 1.00 | 0.68 | 1.00 |
| rs7677766 | A/G | AA/AG/GG | 74/290/372 | 63/339/349 | 0.07 | 0.72 | 0.001 | 0.0004 | 0.002 | 0.00006 | 0.00002 | 0.00004 | 0.00008 | 0.00009 |
| rs17165034 | A/G | AA/AG/GG | 38/233/465 | 31/224/496 | 0.42 | 0.97 | 0.001 | 0.0007 | 0.0002 | 0.00008 | 0.00009 | 0.00006 | 0.00008 | 0.00009 |
| rs9328758 | C/T | CC/CT/TT | 141/343/212 | 176/369/206 | 0.62 | 0.32 | 0.004 | 0.008 | 0.001 | 0.0007 | 0.00007 | 0.00004 | 0.00008 | 0.00009 |
| rs11248042 | C/T | CC/CT/TT | 111/339/266 | 114/333/304 | 0.18 | 0.72 | 0.001 | 0.0004 | 0.002 | 0.00006 | 0.00002 | 0.00004 | 0.00008 | 0.00009 |
| rs11248043 | A/G | AA/AG/GG | 76/357/301 | 114/327/310 | 0.02 | 0.72 | 0.004 | 0.008 | 0.001 | 0.0007 | 0.00007 | 0.00004 | 0.00008 | 0.00009 |
| rs7376690 | A/G | AA/AG/GG | 301/325/475 | 35/226/490 | 0.93 | 0.72 | 0.001 | 0.0004 | 0.002 | 0.00006 | 0.00002 | 0.00004 | 0.00008 | 0.00009 |
| rs6832751 | A/G | AA/AG/GG | 28/234/474 | 23/215/513 | 0.26 | 0.72 | 0.001 | 0.0004 | 0.002 | 0.00006 | 0.00002 | 0.00004 | 0.00008 | 0.00009 |
| rs10155482 | C/A | CC/CA/AA | 37/224/475 | 35/226/490 | 0.93 | 0.72 | 0.001 | 0.0004 | 0.002 | 0.00006 | 0.00002 | 0.00004 | 0.00008 | 0.00009 |

### Table 2. Gene-interaction of MIR137 with CPLX1 between schizophrenia and healthy control groups.

| SNP ID | Interactions | Case | Control | P-value | P-value |
|--------|--------------|------|---------|---------|---------|
| rs2242237 | −0.002 | −0.0003 | 0.59 | 1.00 |
| rs2306251 | −0.002 | −0.0003 | 0.31 | 1.00 |
| rs1172977 | −0.00006 | −0.004 | 0.11 | 1.00 |
| rs7677766 | −0.003 | −0.001 | 0.37 | 1.00 |
| rs17165034 | −0.001 | −0.00007 | 0.49 | 1.00 |
| rs9328758 | −0.0007 | −0.0004 | 0.97 | 1.00 |
| rs11248042 | −0.002 | −0.008 | 0.11 | 1.00 |
| rs11248043 | −0.004 | −0.003 | 0.05 | 0.68 |
| rs7376690 | −0.00008 | −0.0002 | 0.82 | 1.00 |
| rs6832751 | −0.0007 | −0.0009 | 0.65 | 1.00 |
| rs10155482 | −0.001 | −0.001 | 0.72 | 1.00 |
In conclusion, our findings do not support MIR137 and CPLX1 conferring susceptibility to schizophrenia in Han Chinese. Further investigations are warranted to validate our results and identifying the polygenic effects of MIR137 with its downstream target genes in the pathophysiology of schizophrenia.

Methods
Subjects. All procedures were reviewed and approved by Institutional Review Boards of Shanghai Mental Health Center and other participating institutions. This study was performed in accordance with the guidelines laid out in the Declaration of Helsinki as revised in 1989. All subjects provided written informed consent before any study-related procedures were performed.

A total of 736 schizophrenia patients were recruited three mental hospitals in Eastern China, including Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Jinhua Second Hospital and Wenzhou Kangning Hospital. The inclusion criteria for this study were according to our previous publications, and all patients (1) met the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for schizophrenia; (2) were not first-episode; (3) had no chronic physical disease or other psychiatric disorder aside from schizophrenia. Prior to analysis, all diagnosis and review of psychiatric case records were independently checked and verified by two senior psychiatrists. The schizophrenia patients were matched with 751 control subjects enrolled from the hospital staff and students of the School of Medicine in Shanghai, all of which were interviewed by a specialized psychiatrist using the Structured Clinical Interview for DSM-IV-TR Axis I Disorders-Patient Edition (SCID-P) to determine that they had no psychiatric disorders. Any healthy controls found to have any psychiatric disorder or chronic physical disease were excluded from this analysis. The patient and control groups were matched demographically, except education. Detailed participant information was summarized in Supplementary Table S1. All subjects in both the patient and control group were of Han Chinese origin.

SNP selection. We retrieved CHB data from the HapMap database (http://www.hapmap.org) and defined linkage disequilibrium (LD) blocks using Haplovlew 4.2 (Broad Institute, Cambridge, MA, USA) to set inclusion criteria for tagging SNPs. Haplotype-tagging single nucleotide polymorphisms (htSNPs) with r^2 cutoff >0.8 and minor allele frequency (MAF) >0.1 were selected. In total, eleven tag SNPs of CPLX1 were captured for genotyping, including rs2242237, rs2306251, rs11722977, rs7677766, rs17165034, rs9328758, rs11248042, rs11248043, rs7376690, rs6832751 and rs10155482 (Supplemental Table S2).

Genotyping. Genomic DNA of all participants was extracted from peripheral blood using a Tiangen DNA Isolation Kit (Tiangen Biotech, Beijing, China). SNP rs1625579 in MIR137 and 11 htSNPs in CPLX1 were genotyped with the Improved Multiplex Ligase Detection Reaction (IMMLDR) method described in our previous study, with technical support from the Center for Human Genetics Research, Shanghai Genesky Biotech Co., Ltd. The technicians performing genotyping were blind to the study participants. Ten percent of the samples were later randomly selected for duplicate genotyping, which produced 100% concordance.

Brain eQTL analysis for CPLX1 expression. It is known that schizophrenia originates from brain structural and functional abnormalities, and dysregulation of gene expression has a key role in the pathogenesis of this disease. In this study, we performed an eQTL analysis to detect whether CPLX1 is differentially expressed in brain between patients with schizophrenia and healthy controls, using SZDB database (http://www.szdb.org/), a newly developed comprehensive resource for schizophrenia research.

PGC data analysis. To further validate the association between the CPLX1 and schizophrenia, we extracted the schizophrenia genetic association data from the Psychiatric Genomics Consortium (PGC, http://www.broadinstitute.org/mpg/ricopilli/) database and reanalyzed the data set as an independent sample.

Statistical analysis. Demographic data were analyzed using chi-squared or t-test as appropriate. Single marker and gene interaction analyses were conducted using SHEsisPlus (http://shesisplus.bio-x.cn/) and Quanto 1.2.3 (http://hydra.usc.edu/GxE). All statistical analyses were carried out by using the SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). Criterion for statistical significance was set at α = 0.05 and results were two-tailed.

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Acknowledgements
We are deeply grateful to all participants. This work was supported by the National Natural Science Foundation of China (81471358), the Shanghai Science and Technology Commission Foundation (14411969000), the Shanghai Municipal Education Commission—Gaofeng Clinical Medicine Grant Support (20152530), the Shanghai Municipal Commission of Health and Family Planning Foundation (201540029) and the Shanghai Mental Health Center Foundation (2014-FX-03).
Author Contributions
W.L. and C.Z. contributed to the overall design of the study. Y.Z. and X.F. selected the SNPs and wrote the protocol for the genotyping. W.L., Y.Z., W.F., W.T., J.C. and L.S. got involved sample collection. W.L. undertook the statistical analysis and interpretation of data. W.L. carried out the majority of the study under the supervision of C.Z. W.L. and C.Z. wrote the manuscript. All authors contributed to have approved the final manuscript.

Additional Information
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-017-15315-7.

Competing Interests: The authors declare that they have no competing interests.

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