Genetic Effects of DISC1 and G72 (DAOA) on Visual Learning of Patients with Schizophrenia

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Background: Visual learning plays an important role in general populations and patients with schizophrenia. Genetic influences on visual learning remain unknown. Two functional single nucleotide polymorphisms (SNPs), Ser704Cys of the DISC1 gene and M24 (rs1421292) of the G72 gene, are strongly associated with pathogenesis and pathophysiology of schizophrenia. This study examined these two SNPs’ effects on visual learning in schizophrenia patients.

Methods: Two hundred seventy-one patients (mean age, 37.0 years [SD = 9.3]; 159 men) with chronic schizophrenia were genotyped for the DISC1 Ser704Cys and G72 M24 SNPs and assessed for visual learning with Visual Reproduction II (delayed reproduction) of Wechsler Memory Scale – III (WMS-III). For comparison, verbal learning (using Word list II of WMS-III) and attention (by Continuous Performance Test) were also measured.

Results: The DISC1 Ser carriers excelled DISC1 Cys/Cys homozygotes in visual learning (p=0.004, effect size: 0.43), but not in other cognitive functions. G72 M24 A-allele carriers and G72 M24 T/T homozygotes performed similarly (effect size: 0.07). In SNP-SNP interaction analysis, the patients with Ser carrier_T/T had better visual learning than those with Cys/Cys_T/T (p=0.004, effect size: 0.70) and those with Cys/Cys_A-allele carrier (p=0.003, effect size: 0.65). Education had a positive effect (p=0.007), while negative symptoms had a negative effect (p=0.001) on visual learning.

Conclusion: The findings suggest that genetic variations in DISC1 Ser704Cys and G72 M24 affect visual learning in schizophrenia patients. The effect sizes of SNP-SNP interaction surpassed the sum (0.50) of effect sizes from two individual genes, suggesting synergistic DISC1-G72 interaction.

Keywords: attention, DISC1, G72, visual and verbal learning, schizophrenia

Introduction

Visual learning plays an important role in daily life, while schizophrenia is a debilitating disorder with significant visual learning dysfunction. Visual learning, one of the seven cognitive domains (speed of processing, attention/vigilance, working memory, verbal learning, visual learning, reasoning and problem solving, and social cognition) of NIMH-recommended MATRICS (Measurement and Treatment Research to Improve Cognition in Schizophrenia), has gained growing attention for search of endophenotypes of schizophrenia. Three stages of visual learning/memory include encoding of visual perception, visual memory consolidation, and visual memory retrieval. Episodic visual memory encoding, involving visual attention and elaboration, relies heavily on the frontal lobes and visual association cortices. Visual memory consolidation depends mainly on the...
right medial-temporal lobe (MTL), particularly the hippocampus. The right MTL recapitulates the visual learning associated activation pattern and strengthens connections across the relevant lateral cortices. MTLs and lateral cortices are necessary for retrieving unconsolidated and consolidated visual memories, respectively. Visual memory impairments, mediated by organizational deficits, reflect prefrontal cortex mediated executive dysfunction in patients with schizophrenia, while deficits of visual perception are related with their premorbid social functioning. Illness severity and chronicity. Cits of visual perception are related with their premorbid social functioning, illness severity and chronicity. Of note, in patients with schizophrenia, visual memory declines with duration of illness. In addition, both verbal and visual memory abnormalities are well-established features of the schizophrenia spectrum, such as schizotypal personality disorder. Interestingly, visual memory and verbal memory show distinct generational trajectories and visual memory may offer a better potential than verbal memory to predict future risk of developing the disease. Further, visual memory deficits are related with anhedonia in schizophrenia patients, but not with that in psychotic bipolar patients or healthy controls. However, studies exploring visual learning/memory have been far less extensive and consistent than the studies on verbal memory.

The disrupted-in-schizophrenia (DISC1) gene, disrupted by the chromosome 1 breakpoint of a balanced t(1;11) translocation, and G72 (also known as D-amino acid oxidase activator; DAAO) have both been strongly associated with schizophrenia and hippocampal dysfunction. DISC1 is expressed in neurons and glial cells, and greatly impacts the neurodevelopmental processes including neuritic outgrowth, neuronal migration, synaptogenesis, and glutamatergic transmission. Glutamate synapse activation leads to N-methyl-D-aspartic acid receptor (NMDAR) triggered long-term potentiation. Reduced expression of DISC1 binding patterns in postmortem frontal and parietal cortices and hippocampus of schizophrenia patients further implicates the role of DISC1 in schizophrenia pathogenesis. Altered DISC1 expression in orbitofrontal cortex, linking to dorsolateral prefrontal cortex (DLPFC) and verbal working memory, has been reported in schizophrenia. DISC1 genetic variance was associated with neurocognitive dysfunctions, such as deficits in the P300 event-related potential, verbal working memory, verbal attention, and verbal memory, but not yet visual learning/memory in schizophrenia. One study reported the association between DISC1 haplotype, HEP3 (containing two SNPs, T-A allele at rs751229 and rs3738401), and poor short-term visual attention and visual working memory. A common DISC1 nonsynonymous SNP at amino acid 704 (Ser704Cys, rs821616) leading to a serine-to-cystine substitution is associated with altered hippocampal structure and function, impaired verbal working memory, and increased risk for schizophrenia. DISC1-Cys allele was also associated with reduced hippocampal grey matter volume and impaired cognitive ability in women. Since visual memory consolidation depends mainly on the right MTL, particularly the hippocampus, the potential influence of the DISC1 SNP at amino acid 704 on visual memory deserves study too.

Another susceptibility gene, G72, modulates NMDAR neurotransmission via activating D-amino acid oxidase (DAAO) to oxidize D-serine, a potent NMDAR agonist, to hydroxy-pyruvate. Hence, G72 expression with DAAO activation results in decreased NMDAR neurotransmission and schizophrenia phenotype presentation. However, G72 regulation for DAAO activity requires further elucidation. Schizophrenia is strongly associated with markers in the 3’ region of G72, especially marker M24 (rs1421292). G72 genetic abnormalities lead to altered D-serine metabolism and NMDAR neurotransmission. G72 regulates prefrontal synaptic dopamine and is upregulated in postmortem DLPFC of schizophrenia patients. T-allele of M24 compared to A-allele predicts cognitive impairments and altered cortical activity. T/T genotype of G72 M24 genetic variance has been associated with decreased hippocampal activation, increased frontal lobe activation during episodic memory encoding and retrieval, verbal fluency tasks and verbal sentence completion tasks in both healthy and high-risk individuals. G72 M24 genetic variances also affect hippocampal complex and prefrontal cortex function in high-risk individuals. As aforementioned, since visual memory consolidation depends mainly on the hippocampus, the potential influence of G72 M24 genetic variance on visual memory deserves study too.

Structural and functional studies have converged on the potential regulatory effects of DISC1 and G72 on verbal learning/memory. DISC1 genetic variances, not including the Ser704Cys SNP, have been associated with visual attention, while there is no study on G72 and visual learning/memory. Hence, whether DISC1 and G72
influence visual learning/memory in schizophrenia patients deserves further study. To our knowledge, this is the first genetic study on visual learning in humans, aiming to investigate the role of Ser704Cys SNP (rs821616) of DISC1 and M24 SNP (rs1421292) of G72 in neurocognition, with emphasis on visual learning/memory, of patients with schizophrenia.

Methods
Subjects
The study was approved by institutional review board of China Medical University Hospital, Taiwan and conducted in accordance with the current revision of the Declaration of Helsinki.

Han Chinese schizophrenia patients were recruited with the inclusion criteria of 1) having sufficient education to communicate effectively and complete the assessments of the study; 2) being interviewed by experienced research psychiatrist with the Structured Clinical Interview for DSM-IV to confirm the diagnosis of schizophrenia and exclude other psychiatric disorders, including personality disorders and mental retardation; 3) aged 18–65; 4) physically healthy determined by physical examination and laboratory tests (normal blood routine and biochemical tests); and 5) keeping stable dosages of antipsychotic treatment for at least 2 months before enrollment. Patients were excluded when they currently 1) presented with other comorbid psychiatric disorders, substance use disorder or mental retardation; 2) had other existing physical or neurological illnesses; and 3) failed to cooperate with the study.

The participants were 271 unrelated patients with chronically stable schizophrenia, 159 men and 112 women, with a mean age of 37.0 years (SD = 9.3), a mean education level of 11.2 years (SD = 2.4), and a mean duration of illness of 162.0 months (SD = 102.2). After complete description, all participants provided written informed consent.

Clinical Assessments
The Positive and Negative Syndrome Scale (PANSS) was used to assess the clinical symptoms. Clinical ratings were performed by trained and experienced research psychiatrists. Inter-rater reliability was analyzed with the ANOVA test. Only raters reaching intra-class correlation coefficients of ≥0.90 during pre-study training were allowed for rating. Raters met at least once a month for training and reliability re-testing to maintain high interrater reliability and prevent rater drift.

Measurement of Neurocognitive Function
Patients’ cognitive functions, including verbal learning, visual learning, and attention were measured. Word listing II of Wechsler Memory Scale – III (WMS-III) was applied to measure “verbal learning”; Visual Reproduction II of the WMS-III was used for “visual learning”; and the d’ value of Continuous Performance Test (CPT) was employed to assess “sustained attention.”

In “Word list I” of WMS-III, the subjects recalled immediately after a word list of 12 items was read to them each time for four times, and the Word list I score was the number of items correctly recalled after four repeats of the 12 items. A second “interfering” word list without any of the previous 12 items was introduced to the subjects and subjects needed to recall the words from the first word list. In “Word list II,” the subjects recalled as much of the first word list after a 30-min interval since the first list had been initially introduced, and the Word list II score was the number of items correctly recalled after the 30-min interval.

In “Visual Reproduction I,” subjects drew a figure 10 s after the figure was shown to them. Subjects were shown a total of 7 figures. The first 3 figures were shown with a one figure per page display and the remaining 4 figures with a 2 figures per page display. In the “Visual Reproduction II or delayed visual reproduction,” the subjects recalled and drew the figures 30 mins later.

Genotyping
For determining the genotype of DISC1-Ser704Cys SNP (rs821616) from venous blood samples, polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis procedures were applied with DISC1-F (AGGCCATGTGAAAAGGACAG) and DISC1-R (GTCTC AGCTGCAAGTGTC). PCR conditions were: 95°C for 4 min initial heating, followed by 35 cycles of 95°C for 30 sec, 61°C for 30 sec, and 72°C for 30 sec; and finally, 72°C for 7 min. Subjects with Cys/Cys-DISC1 allele gave rise bands of 294 base pairs (bp); Ser/Ser allele, 225 bp and 69 bp; and Ser/Cys allele, 294 bp, 225 bp and 69 bp. The G72 M24 SNP genotyping was performed using the Taqman SNP genotyping assay (ABI: Applied Biosystems Inc., Foster City, CA, USA). The primers and probes of SNPs were from ABI Company. The PCR reaction was conducted in 15µL reaction volume, containing 0.4 µL.
DNA sample (50 ng), 7.5 μL Master mix (Roche), and 0.4 μL 40x primer pairs and probes. A pre-incubation at 95°C for 10 min was employed to activate the Hot-Start DNA polymerase and denature DNA and was followed by 40 amplification cycles of 92°C denaturation for 15 sec, and 60°C for 60 sec. The probe fluorescence signal detection was performed using the ABI Prism 7500 Real-Time PCR System. The results were analyzed with SDS software 2.0 using the allelic discrimination assay program.

Data Analysis
All statistical analysis was carried out with Statistical Package for the Social Science (SPSS), version 22.0 for windows. Deviation of the genotype counts from the Hardy–Weinberg equilibrium was tested using a Chi-square goodness-of-fit test. Demographic and clinical characteristics and cognitive function of patients among genotypes were compared by Chi-square test, Fisher’s exact test, independent sample t test or one-way ANOVA where appropriate. Here, since the number of subjects homozygous for Ser allele was small (n= 5), we grouped Ser carriers together; that is, DISC1-Ser/Ser and DISC1-Ser/Cys patients were combined as DISC1-Ser carriers to compare with DISC1-Cys/Cys homozygotes.24 Meanwhile, A-allele carriers of G72 M24 (A/A and A/T) were combined and compared to G72 M24-T/T homozygotes.16

Multiple regressions were used to compare neurocognitive functions between DISC1-Ser carrier and DISC1 Cys/Cy homozygotes, between G72 M24-A carriers and G72 M24-T/T homozygotes, and among four diplotype groups (1. DISC-Ser carrier with G72-M24 T/T, 2. DISC1-Ser carrier with G72 M24-A carrier, 3. DISC1-Cys/Cys with G72 M24-T/T, and 4. DISC1-Cys/Cys with G72 M24-A carrier) while controlling confounding factors, such as age, onset age, typical/atypical antipsychotics, education years, PANSS-Positive subscale score and PANSS-Negative subscale score. Age,31 onset age,32 illness duration,33 typical/atypical antipsychotic,34 education years35 and active symptoms2 have been associated with effects on cognitive function and visual learning/memory.

Results
Genotype Distribution
The genotype distribution of the DISC1-Ser704Cys SNP was Cys/Cys in 212 patients and Ser carrier in 59 patients (including 54 Ser/Cys and 5 Ser/Ser 59 patients). The distribution was in accordance with Hardy–Weinberg equilibrium ($\chi^2 = 0.66$, d. f.= 1, p > 0.05). The frequency (98%) of Cys allele was similar to those of other Han Chinese studies,17,36 but higher than that (69.2%) in Caucasian populations.22

Regarding the genotype distribution of G72 M24 SNP, there were 161 A-allele carriers (48 AA and 113 TA) and 110 TT homozygotes. The A allele frequency (59%) was lower than that of the Japanese population (75%),37 but higher than that (44%) in Caucasian populations.38 This is the first study exploring G72 M24 SNP on Han Chinese patients with schizophrenia.

Cognitive Functions Among Genotype Groups
As shown in Figure 1, the three DISC1 genotypes showed an allele-dose effect on Visual Reproduction II performance, while the three G72 M24 genotypes had no significant influence. Univariate regression was used for comparison among the three genotype groups: compared to the performance (4.7 ±2.6) of the patients who carried no Ser allele (Cys/Cys homozygotes), those who carried 1 Ser allele (Ser/Cys heterozygotes) had better visual learning ability (5.8±2.7, t=2.593, P=0.010) and those who carried 2 Ser alleles (Ser/Ser homozygotes) also tended to perform better (6.8±3.9, t=1.735, P=0.084), albeit insignificantly perhaps due to the very small sample size of the Ser/Ser group (n=5). We, therefore, grouped Ser carriers (DISC1 Ser/Ser and DISC1 Ser/Cys patients) as a group to compare with DISC1 Cys/Cys homozygotes;24 meanwhile, A-allele carriers of G72 M24 (A/A and A/T) were combined and compared to G72 M24 T/T homozygotes (Table 1).16

Demographic characteristics, clinical manifestations, CPT, and Word list II between DISC1 genotypes (Cys homozygotes vs Ser carriers) and between G72 M24 genotypes (TT homozygotes vs A carriers) were similar (Table 1). Of note, the Ser carriers excelled Cys homozygotes in Visual Reproduction II performance (t= 2.912, p=0.004), while TT homozygotes and A carriers performed similarly (Table 1).

Cognitive Functions Among Diplotype Groups
Demographic characteristics, clinical manifestations, CPT, and Word list II among the 4 diplotype groups (Ser carrier_TT, Ser carrier_A carrier, Cys/Cys_TT, Cys/Cys_A carrier) were similar (Table 2). However, the four diplotype groups differed significantly in Visual Reproduction II performance (F= 3.621, p=0.014) (Table 2).

Moreover, Cys/Cys_T/T group (4.7±2.5, p=0.004) and Cys/Cys_A carrier group (4.8±2.7, p=0.003), yet not Ser...
carrier_A carrier group (5.5±2.3, p=0.125), had poorer Visual Reproduction II scores when compared to Ser carrier_T/T group (6.6±3.1) by univariate regression (Figure 1). Furthermore, after controlling demographic and clinical variables (Table 3) using multiple regression, education duration had positive effect (p=0.007), while negative symptoms (p<0.001), Cys/Cys_T/T (p=0.010) diplotype, and Cys/Cys_A carrier (p=0.002) diplotype had negative effects on Visual Reproduction II scores (Table 3).

Synergistic Effect of DISC1 Ser704Cys and G72 M24 SNP on Visual Learning

We then evaluated genetic effects on delayed visual memory by the effect size of the two SNPs and their combination on delayed visual memory. The effect size on delayed visual memory for each SNP was calculated using Cohen's d, and the effect size for the combination was calculated using Fisher's combined probability test. The results showed that the combination of DISC1 Ser704Cys and G72 M24 SNP had a significant effect on delayed visual memory (p<0.01).

Table 1 Demographic Characteristics, Clinical Features, and Cognitive Functions of Schizophrenia Patients with DISC1 Ser704Cys and G72 M24 Genotypes

<table>
<thead>
<tr>
<th>Variable</th>
<th>DISC1</th>
<th>G72 M24</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cys/Cys</td>
<td>Ser Carrier</td>
</tr>
<tr>
<td>N</td>
<td>212</td>
<td>59</td>
</tr>
<tr>
<td>Sex (F/M), No.</td>
<td>82/130</td>
<td>30/29</td>
</tr>
<tr>
<td>Age, y, mean (SD)</td>
<td>36.8 (9.3)</td>
<td>37.8 (9.4)</td>
</tr>
<tr>
<td>Education, y</td>
<td>11.2 (2.4)</td>
<td>11.1 (2.5)</td>
</tr>
<tr>
<td>Age at illness onset, y</td>
<td>23.3 (6.3)</td>
<td>23.3 (6.4)</td>
</tr>
<tr>
<td>Illness duration, m</td>
<td>161.3 (101.9)</td>
<td>164.5 (104.1)</td>
</tr>
<tr>
<td>Atypical/Typical Antipsychotics/Drug naive</td>
<td>150/57/5</td>
<td>44/13/2</td>
</tr>
<tr>
<td>PANSS-Positive</td>
<td>20.2 (4.6)</td>
<td>19.9 (4.7)</td>
</tr>
<tr>
<td>Negative</td>
<td>23.7 (5.5)</td>
<td>24.0 (4.7)</td>
</tr>
<tr>
<td>CPT</td>
<td>1.54 (1.6)</td>
<td>1.3 (1.5)</td>
</tr>
<tr>
<td>Word list II</td>
<td>4.8 (3.2)</td>
<td>4.9 (2.8)</td>
</tr>
<tr>
<td>Visual II</td>
<td>4.7 (2.6)</td>
<td>5.9 (2.7)</td>
</tr>
</tbody>
</table>

Notes: Bold values indicate statistical significance, **P<0.01. *Chi-square test, Independent sample T test (T-Test), Fisher’s exact test
Abbreviations: PANSS-Positive, Positive and Negative Syndrome Scale-Positive subscale; PANSS-Negative, Positive and Negative Syndrome Scale-Negative subscale; CPT, Continuous Performance Test (of value); Word list II, Word list II of Wechsler Memory Scale – III (WMS-III) (standardized score); Visual II, Visual Reproduction II of WMS-III (standardized score).
visual memory for DISC1 Ser704Cys SNP was 0.43 and for G72 M24 SNP was 0.07 (Figure 2). The additive effect size of both SNPs was 0.50 (= 0.43 + 0.07). Effect sizes of Cys/Cys_T/T (0.70) and Cys/Cys_A-allele (0.65) on delayed visual memory were greater than the additive effect size (0.50) (Figure 2). This implicated that the effect of DISC1 Ser704Cys and G72 on visual learning appeared to be synergistic.

### Discussion

The first major finding of this study is that DISC1-Ser carriers performed better than DISC1-Cys/Cys homozygotes in visual learning (as represented by delayed visual reproduction), but not in verbal learning or attention. Secondly, the patients with the diplotype of DISC1-Ser carrier_G72 M24-T/T surpassed DISC1-Cys/Cys_G72 M24-T/T or DISC1-Cys/Cys_G72 M24-A carrier in visual learning, with a larger effect size.

### Table 2 Demographic Characteristics, Clinical Features, and Cognitive Functions of Schizophrenia Patients with Different DISC1 Ser704Cys and G72 M24 Diplotypes

<table>
<thead>
<tr>
<th>DISC1, G72 M24 Diplotype</th>
<th>Ser Carrier, TT</th>
<th>Ser Carrier, A Carrier</th>
<th>Cys/Cys, TT</th>
<th>Cys/Cys, A Carrier</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>22</td>
<td>37</td>
<td>88</td>
<td>124</td>
<td>0.16*</td>
</tr>
<tr>
<td>Sex (F/M), No.</td>
<td>14/8</td>
<td>16/21</td>
<td>34/54</td>
<td>48/76</td>
<td>0.75b</td>
</tr>
<tr>
<td>Age, y, mean (SD)</td>
<td>38.2 (10.4)</td>
<td>37.5 (8.9)</td>
<td>37.4 (9.6)</td>
<td>36.4 (9.1)</td>
<td>0.12**</td>
</tr>
<tr>
<td>Education, y</td>
<td>11.2 (2.7)</td>
<td>11.0 (2.4)</td>
<td>10.8 (2.2)</td>
<td>11.5 (2.5)</td>
<td>0.12**</td>
</tr>
<tr>
<td>Age at illness onset, y</td>
<td>23.8 (7.6)</td>
<td>22.9 (5.7)</td>
<td>22.7 (5.7)</td>
<td>23.6 (6.6)</td>
<td>0.72b</td>
</tr>
<tr>
<td>Illness duration, m</td>
<td>152.6 (121.3)</td>
<td>171.6 (93.5)</td>
<td>170.7 (105.0)</td>
<td>154.5 (99.5)</td>
<td>0.62b</td>
</tr>
<tr>
<td>Atypical/Typical Antipsychotics/Drug naive</td>
<td>19/3/0</td>
<td>25/10/2</td>
<td>61/24/3</td>
<td>89/33/2</td>
<td>0.60</td>
</tr>
<tr>
<td>PANSS-Positive</td>
<td>19.7 (4.7)</td>
<td>20.0 (4.7)</td>
<td>20.9 (4.9)</td>
<td>19.7 (4.4)</td>
<td>0.25b</td>
</tr>
<tr>
<td>Negative</td>
<td>23.6 (5.2)</td>
<td>24.2 (4.5)</td>
<td>23.8 (4.9)</td>
<td>23.6 (5.8)</td>
<td>0.93b</td>
</tr>
<tr>
<td>CPT</td>
<td>1.0 (1.3)</td>
<td>1.6 (1.7)</td>
<td>1.5 (1.6)</td>
<td>1.6 (1.6)</td>
<td>0.38b</td>
</tr>
<tr>
<td>Word list II</td>
<td>5.0 (3.0)</td>
<td>4.9 (2.8)</td>
<td>4.7 (3.6)</td>
<td>4.8 (2.8)</td>
<td>0.97b</td>
</tr>
<tr>
<td>Visual II</td>
<td>6.6 (3.1)</td>
<td>5.5 (2.3)</td>
<td>4.7 (2.5)</td>
<td>4.8 (2.7)</td>
<td>0.014b,***</td>
</tr>
</tbody>
</table>

Notes: Bold values indicate statistical significance, *P<0.05, **P<0.01, ***P<0.001. Analysis of variance (ANOVA), Fisher’s exact test. Abbreviations: PANSS-Positive, Positive and Negative Syndrome Scale-Positive subscale; PANSS-Negative, Positive and Negative Syndrome Scale-Negative subscale.

### Table 3 Clinical and Genetic Variables Affecting Visual Learning, Represented by Standardized Score of Delayed Visual Reproduction of Wechsler Memory Scale – III

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta (SE)</th>
<th>T</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.012 (0.049)</td>
<td>0.253</td>
<td>0.80</td>
</tr>
<tr>
<td>Education</td>
<td>0.183 (0.068)</td>
<td>2.712</td>
<td>0.007**</td>
</tr>
<tr>
<td>Onset of age</td>
<td>0.043 (0.055)</td>
<td>0.784</td>
<td>0.43</td>
</tr>
<tr>
<td>Illness duration</td>
<td>−0.001 (0.004)</td>
<td>−0.076</td>
<td>0.94</td>
</tr>
<tr>
<td>Antipsychotics, typical/atypical/naive</td>
<td>0.207 (0.355)</td>
<td>0.584</td>
<td>0.56</td>
</tr>
<tr>
<td>PANSS-Positive</td>
<td>−0.029 (0.034)</td>
<td>−0.840</td>
<td>0.40</td>
</tr>
<tr>
<td>PANSS-Negative</td>
<td>−0.127 (0.030)</td>
<td>−4.228</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>Ser Carrier, TT in DISC1, G72 M24 diplotype</td>
<td>0.010*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser Carrier, A-Carrier</td>
<td>−0.815 (0.671)</td>
<td>−1.216</td>
<td>0.23</td>
</tr>
<tr>
<td>Cys/Cys, TT</td>
<td>−1.544 (0.395)</td>
<td>−2.593</td>
<td>0.010*</td>
</tr>
<tr>
<td>Cys/Cys, A-Carrier</td>
<td>−1.774 (0.578)</td>
<td>−3.068</td>
<td>0.002**</td>
</tr>
</tbody>
</table>

Notes: Bold values indicate statistical significance, *P<0.05, **P<0.01, ***P<0.001. Multiple regression was utilized to compare visual learning among schizophrenia patients with different DISC1 Ser704Cys_G72 M24 diplotypes. Education duration had a positive effect on visual learning (p= 0.007), while negative symptoms (p <0.001), Cys/Cys_T/T diplotype (p=0.001), and Cys/Cys_A-carrier diplotype (p=0.002) showed negative associations with visual learning. Abbreviations: Typical, typical antipsychotics; atypical, atypical antipsychotics; naive, antipsychotics naive; PANSS-Positive, Positive and Negative Syndrome Scale-Positive subscale; PANSS-Negative, Positive and Negative Syndrome Scale-Negative subscale.
Visual learning involves the ability to store and retrieve previously experienced visual sensations and perceptions when the stimuli that originally evoked them are no longer present. And one, with intact memory encoding and consolidation, should be able to place in and retrieve memory information that resembles objects, places, animals or people in sort of a mental image from "the mind’s eye." 

Moreover, visual perception and memory often perceive image as a whole and not in pieces and group images according to six features: proximity, similarity, closure, symmetry, common fate and continuity. It is sensitive in ensembling characteristics of complex objects such as face and its impairments are associated with poor social functioning and illness severity in schizophrenia.

Furthermore, visual learning deficits, mediated by organization skill deficits and poor memory encoding, contribute to poor planning skills and executive dysfunction. Patients with schizophrenia often use a piecemeal approach and have difficulty processing the gestalt of a complex visual stimulus in visual learning evaluation. Hence, impaired processing of global features in a local-global paradigm in these patients often results in overemphasis on non-critical features and deletion of available attention resources before they can process the critical features or interpret wholes as meaningful gestalts, which further implies their inefficient organizational strategy. Deficits in visual perception, visual memory encoding and consolidation all contribute to visual learning deficits. Li et al also found that Cys-allele carriers have less efficient information transfer than Ser homozygotes. Cys-allele carriers had a significantly lower global efficiency of their brain networks and decreased white matter integrity.

Animal genetic study has demonstrated a close association of DISC1, cognitive functions, and hippocampal function/structure. Human studies have also shown an association of several DISC1 haplotypes, such as HEP1 (rs6675821, rs1000731, rs3890280), HEP2/HEP3 segment (rs1615409, rs766288, rs751229, rs3738401), with altered cognitive function. Cys allele has been associated with impaired verbal learning/long-term memory in schizophrenia and normal controls; while a few DISC1 haplotypes, but not including the SNP of the current study, for example, HEP2/HEP3 segment (rs1615409, rs766288, rs751229, rs3738401), HEP3 (rs751229, rs3738401) and hCV1650649 were associated with visual attention/search, visual working memory, and visuospatial memory. On the other hand, G72 M24-TT homozygotes were associated with decreased hippocampal activation and increased prefrontal activation in fMRI during episodic memory encoding and retrieval, working memory and verbal completion tasks.

We are the first group to report the association of genetic effects between DISC1 Ser704Cys SNP and G72 M24 SNP and delayed visual memory/learning impairment. Interaction of the SNPs on visual memory was significantly lower global efficiency of their brain networks and decreased white matter integrity. Finding of poorer visual learning and delayed visual memory in Cys allele carriers are consistent with previous
frontal-parietal network (insular cortex) disruption studies in schizophrenia patients, where the insular cortex of the temporal cortex is implied for general consolidation of visual recognition memories. Association of DISC1 Ser704Cys SNP and G72 M24 SNP and visual memory/learning impairment is further supported by the impact of the DISC1 Ser704Cys SNP on visual memory associated brain structural changes and the impact of the G72 M24 SNP on memory task associated brain activations. Cys allele has been associated with reduced supramarginal gyrus volume, which is part of inferior parietal lobe responsible for enactment effect specific for visual memory encoding.

Visual memory recall predominantly activates the left superior frontal gyrus and the intraparietal cortex, whereas recall of visually learned locations activates the bilateral superior parietal cortices. DISC1 and G72 genetic variances are closely associated with these visual-memory-associated brain areas including insular cortex, medial superior frontal and prefrontal gyri, and hippocampus. Ser/Ser homozygote with schizophrenia had similar or larger medial superior frontal gyrus and insular cortex volumes when compared with Cys carriers. Moreover, DISC1 Ser allele homozygotes compared to Cys allele carriers had greater hippocampal gray matter volume, which further supported the possible concept that schizophrenia patients with Cys allele had poorer visual memory (Figure 1).

The study also found that longer education duration benefited visual learning (Table 3). This finding is consistent with previous studies implying that higher Intelligent Quotient (IQ) favors better delayed visual memory performance. This study also found that negative symptoms had detrimental effects on visual learning. Negative symptoms have been associated with visual memory impairment in patients with schizophrenia.

Limitations
This study has several limitations. Firstly, the distribution of Cys and Ser allele is different from the previous study. Frequency of Cys homozygotes is higher, while Ser homozygotes is lower in our study. This may be explained by the Han Chinese ethnicity of the subjects. However, the distribution of the DISC1 genotype is similar to the report of NCBI. Moreover, there is no control group for this study. The reported effects of the studied SNPs on visual memory may be general effects for any subject, which would mean that they are not specific to schizophrenia. Inclusion of a control group in future replication study is needed. Thirdly, we used Word List of WMS-III rather than California Verbal Test to assess verbal memory due to the limited availability of the Chinese version of the California Verbal Test. However, the measures from the two tests were highly correlated. Fourthly, the use of a single neuropsychological test to assess visual learning may be considered a shortcoming of the paper.

Conclusions
The present study is the first genetic study of visual memory in humans. It is also the first to investigate G72 M24 SNP in Han Chinese patients with schizophrenia and the first to explore the relationship between DISC1 Ser 704Cys and G72 M24 genetic effects and visual memory in schizophrenia patients. Study findings indicated that schizophrenia patients with the Cys/Cys_T/T or Cys/Cys_A carrier had poorer visual learning. The combination of the two SNPs also supported an additive effect of the individual SNPs, suggesting that DISC1 and G72 might interact synergistically and thereby accounting for variance in hippocampal structure, function, and activation. Callicot also suggested that the risk of developing schizophrenia and associated cognitive deficits may be due to haplotypes monitored by Ser704Cys rather than the SNP itself. Thus, investigating the roles of other genetic variants of DISC1 and their interactions with G72 genetic variances may further help search for the endophenotype and subtyping of schizophrenia patients and development of targeted treatments.

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Disclosure
All authors declare that they have no conflicts of interest in this work.

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