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ARTICLE

Genetic associations and expression of extra-short isoforms of disrupted-in-schizophrenia 1 in a neurocognitive subgroup of schizophrenia

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Abstract

Disrupted-in-schizophrenia 1 (*DISC1*) was reported to be associated with schizophrenia. In a previous study, we found significant association with schizophrenia patients with deficient sustained attention assessed by continuous performance test (CPT). This study aimed to identify risk polymorphisms in this specific neurocognitive subgroup and investigate the expression of different isoforms of *DISC1*. A total of 83 genetic variants were identified through direct sequencing in 50 controls and 100 schizophrenia patients. Fourteen variants were genotyped in 600 controls and 912 patients. Patients were subgrouped by familial loading (multiplex or simplex) and performance on CPT. The frequency of AA genotype of rs11122324 at the 3′-UTR of Es and Esv1 isoforms and of rs2793091 at intron 4 were significantly higher in multiplex schizophrenia patients than those in controls (corrected $p < 0.05$). In further subgrouping, the frequency of AA genotype of the two SNPs were significantly higher in multiplex schizophrenia patients with deficient sustained attention than those in controls (corrected $p < 0.005$). The mRNA expression levels of two extra-short isoforms (Es and Esv1) in the EBV-transformed lymphocytes of schizophrenia were significantly higher than those of controls. Luciferase reporter assays demonstrated that the A-allele of rs11122324 significantly upregulated *DISC1* extra-short isoforms transcription compared with the G-allele. We found two SNPs (rs11122324 and rs2793091) of *DISC1* may be specifically associated with multiplex schizophrenia patients with deficient sustained attention. The SNP rs11122324 may be a risk polymorphism, which may have functional influence on the transcription of Es and Esv1 through increasing their expression.

Introduction

Genetic components play an important role in the etiology of schizophrenia based on evidence of family, twin, and adoption studies, with an estimated heritability of 60–80% [1]. Previous genetic linkage studies have identified

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chromosome 1q42 as a candidate region for schizophrenia [2, 3]. A balanced translocation (1; 11) (q42.1; q14.3) disrupting two genes (disrupted-in-schizophrenia 1 [*DISC1*] and *DISC2*) in chromosome 1 was found to be associated with major mental illnesses, including schizophrenia in a large Scottish family pedigree [4]. Although earlier small-scale genetic association studies have reported modestly significant associations between *DISC1* and schizophrenia [5], more recent large-scale meta-analyses [6, 7] and the largest genome-wide association study (GWAS) [8] have concluded that DISC1 is not valid as a common risk gene for the Diagnostic and Statistical Manual of Mental Disorders (DSM)-diagnosed schizophrenia, especially in Caucasian ancestry. A recent meta-analysis [9], containing a larger Chinese and Japanese population than in previous studies and including 31 eligible studies with 13 markers in *DISC1* (the maximal case number was 9031 and control number 9733 for rs821616) suggested that *DISC1* polymorphisms (rs821616 and rs821597) increased schizophrenia risk in overall populations and especially in Chinese populations.

Although DISC1 is not associated with DSM-diagnosed schizophrenia, accumulating evidence has been reported in association between DISC1 and specific endophenotypes that are commonly associated with schizophrenia and other major mental disorders, including some neurocognitive endophenotypes (such as sustained attention [10], working memory [11, 12]), electrophysiological endophenotypes (such as auditory-event-related potential [13]), and neuroimage endophenotypes (such as reduced gray matter volume of hippocampus [14], prefrontal cortex [12], cingulate and several other brain regions [15], and white matter integrity [16]).

DISC1 is an intracellular hub for protein/RNA networks, involving the function for early neurodevelopment and synaptic regulation [17], including progenitor proliferation [18], neuronal migration [18, 19], neurites outgrowth [20], synaptic formation and regulation [21, 22], and neuronal signaling [22, 23]. Many lines of evidence have indicated that deficits of these neuronal processes, in particular those in early development, underlie circuitry and behavioral endophenotypes relevant to a wide range of mental illness [24]. Several *DISC1* interactors have also been defined as independent genetic susceptibility factors for psychiatric illness [25]. Various *DISC1* transgenic animal models coupled with different environmental stressors, have proven successful in satisfying face validity as models of a wide range of human psychiatric conditions [26].

The alternative splicing of *DISC1* in the human brain is exceptionally complex, with more than 50 splice variants in different stages of brain development [27]. However, results of studies of the mRNA level of total amount of different *DISC1* isoforms were inconsistent between schizophrenia patients and controls. One study reported no significant differences in the dorsal lateral prefrontal cortex [28], another reported significantly increased total *DISC1* in the mononuclear cells of peripheral blood in drug-naive schizophrenia patients [29], and the other study reported significantly decreased *DISC1* in the whole blood of schizophrenia patients [30]. An extensive study of mRNA levels of different *DISC1* isoforms found that the expressions of three isoforms, including two short isoform variants and an extra-short isoform variant (Esv1), were significantly higher in the hippocampus of patients with schizophrenia [27].

Although most GWAS and meta-analyses showed negative results for associations between *DISC1* and schizophrenia, several factors motivated our group to study this candidate gene. First, as shown in the most recent meta-analysis including more Chinese and Japanese subject [9], ancestral differences may influence the association evidence of this gene. Second, *DISC1* is reported to be associated with some neurocognitive and neuroimaging features of schizophrenia, and is significantly associated with specific subgroups of schizophrenia, which tests the hypothesis that this gene may be specifically associated with certain subgroups of schizophrenia or neurocognitive endophenotypes. Third, convergent functional genomics approaches that integrate genetic, gene expression, and functional data continue to support the involvement of *DISC1* in schizophrenia and related biological pathways [31].

In our previous study [10], we found significant associations between *DISC1* and a subgroup of schizophrenia patients with deficient sustained attention assessed by the continuous performance test (CPT), using 102 families with at least two siblings affected with schizophrenia. In the present study, we aimed to re-sequence the genomic regions of this gene and to study the associations between the identified single-nucleoride polymorphisms (SNPs) and the subgroup of schizophrenia patients with deficient sustained attention, and to study the mRNA expression of different isoforms of *DISC1*.

Methods

Subjects

Subjects for direct sequencing

Study subjects included the probands from a project, in which schizophrenia probands from both simplex (i.e., only one affected offspring in the two-generation family) and multiplex families (i.e., at least two affected siblings in the family) were recruited from National Taiwan University Hospital and Ju-Shan Psychiatric Hospital from 2002 to 2005. The study protocol was reviewed and approved by

the institutional review boards of the two hospitals. The inclusion criteria were probands meeting the DSM, 4th edition (DSM-IV) diagnostic criteria of schizophrenia and the exclusion criteria were severe neurological abnormality, substance-use disorder except for smoking, intellectual disability, and aboriginal ancestry. Normal controls were recruited with inclusion criteria of no lifetime psychiatric disorders and no family history of schizophrenia in first-degree relatives. Signed informed consent was obtained from all subjects after detailed explanation of the study. A total of 50 independent multiplex patients, 50 simplex patients, and 50 normal controls were selected for direct sequencing study.

Subjects for genotyping

The patients came from two types of families: multiplex families and simplex families. The multiplex families were recruited from two research programs: the first one [32] conducted from 1993 to 2001 and the second one [33] conducted from 1998 to 2002. Protocols of the research projects were reviewed and approved by the institutional review board of National Taiwan University Hospital. Signed informed consent was obtained from family members after the procedures had been fully explained. The 93 families of the first research program were interviewed by research psychiatrists using the Psychiatrist Diagnostic Assessment [34]. The 609 families of the second research program were interviewed by well-trained assistants using the Mandarin Chinese version of the Diagnostic Interview for Genetic Studies [35]. For both studies, the final research diagnosis was formulated by integrating interview data with clinical information from medical records, based on DSM-IV criteria. To assure the independence of multiplex subjects, only one affected sibling was randomly selected from each multiplex family. A total of 702 patients from these multiplex families were included in this study. A total of 87 independent patients from the multiplex families were included in our previous family-based association [10]. The simplex families were recruited from another independent project [36]. The diagnostic assessment was the same as that for the second research project. A total of 210 simplex families were recruited. Therefore, a total of 912 schizophrenia patients comprised the analytic sample for this genotyping study.

The normal controls were selected from a representative Taiwanese supernormal genomic sample [37] with the inclusion criteria of age over 60 years and a score above 14 on the Short Portable Mental Status Questionnaire. Control subjects were recruited by the Institute of Biomedical Science, Academia Sinica. A total of 600 supernormal control individuals (313 males and 287 females) were included in this study.

Subjects for gene expression study

The subjects for the mRNA expression study were from the same project as the direct sequencing study. A total of 85 patients and 36 controls were included in this gene expression study. The mean age of controls was 36.9 (±10.7) years and 41.7% were male. The mean age of patients was 33.0 (±9.1) years and 58.8% were male. No significant differences were found in the age and gender of patients and controls.

Methods

Direct sequencing

All exons, promoter regions, and the associated haplotype regions as reported in our previous study [10] were defined using the following bioinformatics process. As the highly conserved regions across different species are more resistant to evolutionary pressure, implying more important for gene function, we also sequenced the highly conserved regions.

The exon regions of *DISC1* were evaluated for all transcripts and variants of expressed sequence tags updated by the Integrated Splicing Variants database [38]. Six isoforms, including long form (L), long form variants (Lv), short form (S), extra-short form (Es), the other two extra-short isoforms, and six expressed sequence tags variants were found in the *DISC1* genomic region (Supplementary Fig. 1). The promoter region was defined as the 2000 base pairs (bp) before the start exon of each transcript. The highly conserved regions were predicted by MultiPipMaker (http://pipmaker.bx.psu.edu/pipmaker/) output results. These conserved regions were arranged into clusters by MultiPipmaker through the percent identity plot among humans, chimpanzees, mice, and rats. The genomic positions of previously significantly associated haplotype regions from rs2793092 to rs2793091 (from intron 3 to intron 4 according to L isofrom) [10] and highly conserved regions were integrated.

The genomic regions of *DISC1* selected from the above bioinformatics procedures were amplified by PCR. PCR products were purified with exonuclease I and shrimp alkaline phosphatase (USB Corporation, Cleveland, Ohio, USA), and sequenced from both ends. DNA-sequencing reactions were performed with BigDye Terminator Cycle Sequencing Version 3.1 (Applied Biosystems, Foster City, CA, USA) followed by analysis on an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

The resulting sequences were compared and aligned using the Polyphred Sequence Alignment Editor (http://droog.mbt.washington.edu/PolyPhred.htm). The reference sequences were obtained from the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/).

SNP genotyping

The selection criteria for further large-scale genotyping of genetic variants identified from direct sequencing are (1) all exon variants; (2) differences in minor allele frequency (MAF) >2% between controls and patients, if the variant frequency is less common (MAF in these subjects was between 0 and 10%); and (3) MAF differences >4% for more common variants (MAF between 10 and 50%). Genotyping was performed using the matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) mass spectrometry. Primers and probes flanking the SNPs were designed using SpectroDESIGNER software (Sequenom, San Diego, CA, USA). A DNA fragment (100–300 bp) encompassing the SNP site was amplified using PCR (GeneAmp 9700 thermocycler, Applied Biosystems, USA) according to the manufacturer's instructions.

After removing the un-incorporated deoxynucleotide triphosphate (dNTP) and inactivating the shrimp alkaline phosphatase from the PCR product, primer extension was performed by adding the probe, Thermo Sequenase (Amersham Pharmacia, Piscataway, NJ, USA), and an appropriate dideoxynucleotide triphosphate/dNTP mixture, followed by 55 cycles of denaturing at 94 °C for 5 s, annealing at 52 °C for 5 s, and extension at 72 °C for 5 s. The other extension products were differentiated by mass through MALDI-TOF.

Real-time PCR (RT-PCR)

The mRNA expressions of different DISC1 isoforms, including total, L, Lv, S, Es, and Esv1 (according to the nomenclature in Nakata et al. [27]), were measured in Epstein–Barr virus (EBV)-transformed lymphoblastic cell lines. Trizol Reagent (Invitrogen Life Technologies) was used according to the manufacturer's guidelines to extract total RNA.

RT-PCR was performed for *DISC1* isoforms and a housekeeping gene, TATA-box binding protein (*TBP*), using pre-designed gene-specific TaqMan® probes and primer sets purchased from Applied Biosystems (Branchburg, NJ). The sequences of primers of different isoforms were listed in Supplementary Table 1. RT-PCR amplification was conducted using Taqman One-Step RT-PCR Master Mix Reagent (Applied Biosystems, Branchburg, NJ) on an ABI StepOne Plus System (Applied Biosystems, Branchburg, NJ), according to the manufacturer's instructions. Gene expression was quantified relative to *TBP* expression using StepOne Software (Applied Biosystems, Branchburg, NJ) and the relative quantification method. The relative expression level of *DISC1* isoforms compared with that of *TBP* was defined as $-\Delta CT = -[CT_{DISC1} - CT_{TBP}]$, where CT was the cycle threshold. The *DISC1* mRNA/*TBP* mRNA ratio was calculated from $2^{-\Delta CT} \times K$, in which K was a constant.

Luciferase reporter assay

To discern the potential effects of different alleles of the 3′-untranlated region (UTR) variant (rs11122324) of *DISC1* Es and Esv1 isoforms, two luciferase reporter vectors were constructed by cloning different alleles (G-allele and A-allele) of rs11122324 in the 3′-UTR region of *DISC1* Es (also Esv1) isoform into pGL4.10[luc2] vector (Promega, Madison City, WI, USA). The cytomegalovirus promoter was ligated to drive the luciferase expression (luc2) and the original 3′-UTR SV40 was replaced with the 3′-UTR sequence of *DISC1* Es (also Esv1) isoform. All inserts were confirmed by direct sequencing. The construction process is displayed in Supplementary Fig. 2. The 3′-UTR fragment with A-allele of rs11122324 was amplified by PCR from the genomic DNA of a patient confirmed to carry this allele. The 3′-UTR fragment with G-allele was obtained by single point mutagenesis (A to G) from the above-described fragment. Therefore, the two vectors were the same, except for the variant of rs11122324 in their 3′-UTR. We transfected three vectors, the first is control vector (pCMV4.10), the G-allele vector (pCMV4.10-DISC1-G), and the A-allele vector (pCMV4.10-DISC1-A) into two cell lines (HEK293T and U251), respectively. Cells were seeded in 24-well plates at a density of 1×10^5 cells per well and cultured in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad City, CA, USA) supplemented with 10% fetal bovine serum. Cells were co-transfected with 1.8 μg of each reporter vector and 0.2 μg of *Renilla* luciferase plasmid (Promega) using Lipofectamine 2000 transfection reagent (Invitrogen). The cells were collected after 72 h. Luciferase activity was immediately detected by the Dual-Luciferase Reporter Assay System (Promega) using a GloMax 96 Luminometer (Promega) and luciferase assay kit (Promega). The experiments were repeated at least three times in both cell lines. Relative luciferase activity is calculated as the ratio of Firefly luciferase activity to *Renilla* luciferase activity. For control of the variations of transfection efficacy in repeated experiments, the relative transcription activities of the A-allele and the G-allele vector were calculated as the ratio of relative luciferase activity of the A-allele or the G-allele vector to that of the control vector (pCMV4.10) in the same experiment.

Sustained attention assessment

A CPT machine from Sunrise System, v. 2.20 (Pembroke, MA, USA) was used to assess sustained attention. The procedure has satisfactory reliability and has been described in detail elsewhere. Each subject undertook two CPT

sessions: the undegraded 1–9 task and the 25% degraded 1–9 task. Subjects were asked to respond whenever the number "9" preceded by the number "1" appeared on the screen. During the 25% degraded session, a pattern of "snow" was used to partially obscure the background and foreground, visually distorting the number. One signal-detection index of performance on the test, sensitivity (d′), was derived from the hit rate (probability of response to target trials) and false-alarm rate (probability of response to non-target trials). Sensitivity (d′) is an individual's ability to discriminate target stimuli from non-target stimuli.

The *z*-score of d′ on CPT was calculated according to age, gender, and education based on a community-based sample of 345 controls [35]. The cut point of d′ was set as −2.5 from our previous family genetic study [39]. In this study, 303 patients had *z*-scores of d′ on undegraded CPT that were below −2.5 (deficit) and 458 patients with scores above −2.5 (non-deficit); 377 patients had *z*-scores of d′ on degraded CPT that were below −2.5 (deficit) and 360 patients with scores above −2.5 (non-deficit).

Statistical analysis

Hardy–Weinberg equilibrium was assessed by using the ALLELE procedure in SAS/GENETICS release 9.4. Allele and genotype frequencies were compared between the case and control groups by using the χ^2-test or Fisher's exact test where appropriate. The false discovery rate method was used to correct for multiple comparisons. The relative expression levels of different *DISC1* isoforms between the case and control groups were compared using the two-sample *t*-test. The relative transcription activities (ratio to the control vector (pCMV4.10)) of the G-allele and A-allele vector were compared using paired *t*-test. A p-value < 0.05 was considered significant.

Results

Genetic association results

A total of 24,854 bp of *DISC1* genomic region have been sequenced. Using direct sequencing, 83 genetic variations have been identified, of which 16 variants are not found in 1000 Genomes or other public variation resources, and 10 are exon variants, of which none is novel. All genetic variations are listed in Supplementary Table 2. Sixteen variations, including 10 exon variants, met the criteria for further genotyping in 600 controls and 912 schizophrenia patients. However, we failed to genotype two exon variants (P193S and R264Q) using our genotyping method. Therefore, a total of 14 SNPs had the genotyping data. All 14 SNPs were compatible with Hardy–Weinberg equilibrium. The detailed information and association results in all samples are listed in Table 1. No significant differences were found between all patients ($N = 912$) and controls in the allele and genotype frequencies for the 14 SNPs. As our previous association results were found to be significant in the multiplex families, patients were further subgrouped into the multiplex patients group ($N = 702$) and the simplex patient group ($N = 210$). The genotype frequency of rs11122324 was found at the 3′-UTR of Es and Esv1 isoform, and that of rs2793091 at intron 4 was significantly different between the multiplex patients and controls (corrected $p = 0.0146$ and 0.0036, respectively). The allele frequencies of the two SNPs were not significantly different after multiple correction. No significant differences were found between the multiplex patients and controls in the allele and genotype frequency for the other 12 SNPs. No significant differences in either allele or genotype frequencies were found between the simplex patients and controls.

Further subgrouping of the multiplex patients into those with and without sustained attention deficit revealed that the genotype frequencies of rs11122324 and rs2793091 were significantly different between the patients with deficient sustained attention and controls (corrected $p = 0.0146$ and 0.0036, respectively, in undegraded CPT and corrected $p =$ 0.0058 and 0.0013, respectively, in degraded CPT), but were not significantly different between the multiplex patients without sustained attention deficit and controls. The allele frequencies of the two SNPs were not significantly different after multiple correction. The detailed genotype distribution and genotype-wise association results of rs11122324 and rs2793091 in the different subgroups are listed in Table 2. The proportions of AA genotype of rs11122324 and rs2793091 (recessive genotype) were observed to be increased in the subgroups of multiplex and deficient sustained attention. The genotype-wise significance became even more evident when compared under the recessive model (AA genotype vs. non-AA genotype) of the two SNPs. The detailed results are listed in Table 2.

We further explored the association between the DISC1 SNPs and sustained attention in schizophrenia patients. We found significant association between the performance of CPT and the genotypes of rs1865225, rs11122324, rs2793091, and rs2492367 (Supplementary Table 3). However, the association results were not significant after multiple correction. Under recessive models of rs11122324 and rs2793091, it showed the patients with AA genotypes of rs11122324 and rs2793091 had significant poorer performance of CPT than those with GG and GA genotypes ($t = 2.430$, d.f. $= 757$, $p = 0.015$ for rs11122324; $t = 2.894$, d.f. $= 706$, $p = 0.004$ for rs2793091).

Table 1 The detail information and association results of the 14 genotyped genetic variants identified from direct sequencing in all samples ($N = 1512$)

SNP name	Chromosome location[a]	Gene position	Allele type[b]	MAF in controls ($N = 600$)	MAF in patients ($N = 912$)	Allele-wise χ^2 (p-value)	Genotype-wise χ^2 (p-value)
rs1865225	231627258	Intron 1	A/G	0.373	0.383	0.27 (0.60)	1.39 (0.5)
rs56020408	231694105	Exon 2 (A116V)	C/T	0.015	0.0099	1.21 (0.27)	1.66 (0.2)
rs113312552	231694517	Exon 2 (D253D)	C/T	0	0.0012	0.12 (0.52)	0.21 (0.52)
rs55795950	231694741	Exon 2 (T328N)	C/A	0.0116	0.0104	0.20 (0.89)	0.11 (0.75)
rs11122324	231723435	3′-UTR of Es and Esv1	G/A	0.37	0.397	2.21 (0.14)	7.27 (0.026)
rs2793091	231758862	Intron 4	G/A	0.47	0.5	2.61 (0.11)	7.65 (0.022)
rs3738402	231767264	Exon 5 (L465L)	C/T	0.22	0.23	0.44 (0.51)	3.3 (0.19)
rs2492367	231770843	Exon 6 (I469I)	C/T	0.173	0.173	0.01 (0.96)	0.58 (0.75)
rs56229136	231771027	Exon 6 (G531R)	G/C	0.045	0.033	2.6 (0.11)	3.52 (0.17)
rs12133766	231818399	Exon 9 (L621L)	G/A	0.031	0.023	1.73 (0.19)	0.45 (0.5)
rs56071042	231822397	Intron 9	G/A	0.361	0.386	1.95 (0.16)	2.06 (0.36)
rs3082	231866646	3′-UTR of S isoform	A/G	0.369	0.375	0.11 (0.74)	0.21 (0.9)
rs821616	232008852	Exon 11 (S704C)	A/T	0.102	0.118	1.92 (0.17)	4.82 (0.09)
rs16856254	232026118	Intron 11	G/T	0.064	0.072	0.66 (0.42)	1.27 (0.53)

[a]The chromosome position and gene was determined based upon the NCBI reference sequence Build 38

[b]Second allele under oblique line (/) is the minor allele.

Table 2 The genotype distribution and genotype-wise association results of rs11122324 and rs2793091 under additive and recessive model in the different subgroups

	Frequency of genotypes of rs11122324					Frequency of genotypes of rs2793091				
	GG	GA	AA	Genotypic model χ^2 (nominal p, corrected p)	Recessive model χ^2 (nominal p, corrected p)	GG	GA	AA	Genotypic model χ^2 (nominal p, corrected p)	Recessive model χ^2 (nominal p, corrected p)
Control ($N = 600$)	0.379	0.502	0.119	N.A.	NA	0.256	0.547	0.197	N.A.	NA
all patients ($N = 912$)	0.374	0.458	0.168	7.52 (0.026, 0.026)	7.11 (0.0089, 0.0089)	0.256	0.485	0.259	7.65 (0.022, 0.022)	6.83(0.009, 0.009)
multiplex patients ($N = 702$)	0.370	0.450	0.179	9.61 (0.0097, **0.0146**)	9.12 (0.0031, **0.0042**)	0.254	0.468	0.277	12.48 (0.0019, **0.0036**)	11.27 (0.00079, **0.0014**)
All AD (undegraded CPT) ($N = 303$)	0.356	0.452	0.191	8.61 (0.013, **0.0156**)	8.54 (0.0035, **0.0042**)	0.247	0.463	0.289	9.86 (0.0072, **0.0086**)	9.33 (0.0023, **0.0028**)
All AD (degraded CPT) ($N = 377$)	0.354	0.447	0.199	11.76 (0.0028, **0.0084**)	11.66 (0.00064, **0.0019**)	0.256	0.446	0.298	14.04 (0.00089, **0.0027**)	12.58 (0.00039, **0.0012**)
Multiplex AD (undegraded CPT) ($N = 254$)	0.358	0.441	0.201	9.84 (0.0073, **0.0146**)	9.66 (0.0019, **0.0038**)	0.254	0.444	0.302	12.05 (0.0024, **0.0036**)	10.94 (0.00094, **0.0014**)
Multiplex AD (degraded CPT) ($N = 297$)	0.360	0.428	0.212	13.89 (0.00096, **0.0058**)	13.44 (0.00025, **0.0015**)	0.254	0.431	0.315	16.89 (0.00022, **0.0013**)	15.23 (0.000095, **0.00057**)

AD sustained attention-deficit patients with z-scores of $d' < -2.5$, *CPT* continuous performance test, *NA* non-applicable

Expression study

The mRNA expression levels of *DISC1* Es and Esv1 isoform in the EBV-transformed lymphocytes of schizophrenia ($N = 85$) were found to be significantly higher than those of controls ($N = 36$) ($t = 2.03$, d.f. $= 119$, $p = 0.047$ for Es isoform; $t = 2.13$, d.f. $= 119$, $p = 0.035$ for Esv1 isoform), but the results became nonsignificant after multiple correction. The mRNA expression levels of total DISC1, L, Lv, S isoforms were not significantly different between patients and controls (Fig. 1a). As rs11122324 was located at the 3′-UTR of Es and Esv1 isoforms, the relationship between the genotypes of rs11122324 and the expression of the two isoforms were explored. Patients with A-allele of rs11122324 (GA and AA genotypes) were found to have significantly higher expression of Es isoform than those with GG genotype (dominant model for A-allele) ($t = 2.01$, d.f. $= 76.9$, $p = 0.048$). There were no significant differences for Esv1 isoform expression under this model. However, there were no significant differences for both Es and Esv1 isoform between patients with AA genotype of rs11122324 and those with GA and GG genotypes (recessive model) (Fig. 1b).

We found there were no significant differences of all the isoforms among the simplex patients, the multiplex patients and control group. There were significantly negative correlations between the performances of CPT and the expression of Esv1 isoform in all samples (Pearson correlation $r = -0.255$, $p = 0.005$, d.f. $= 121$, with the z-scores of undegraded CPT; $r = -0.253$, $p = 0.006$, d.f. $= 121$, with the z-scores of degraded CPT), whereas nonsignificant correlations between the performances of CPT and the

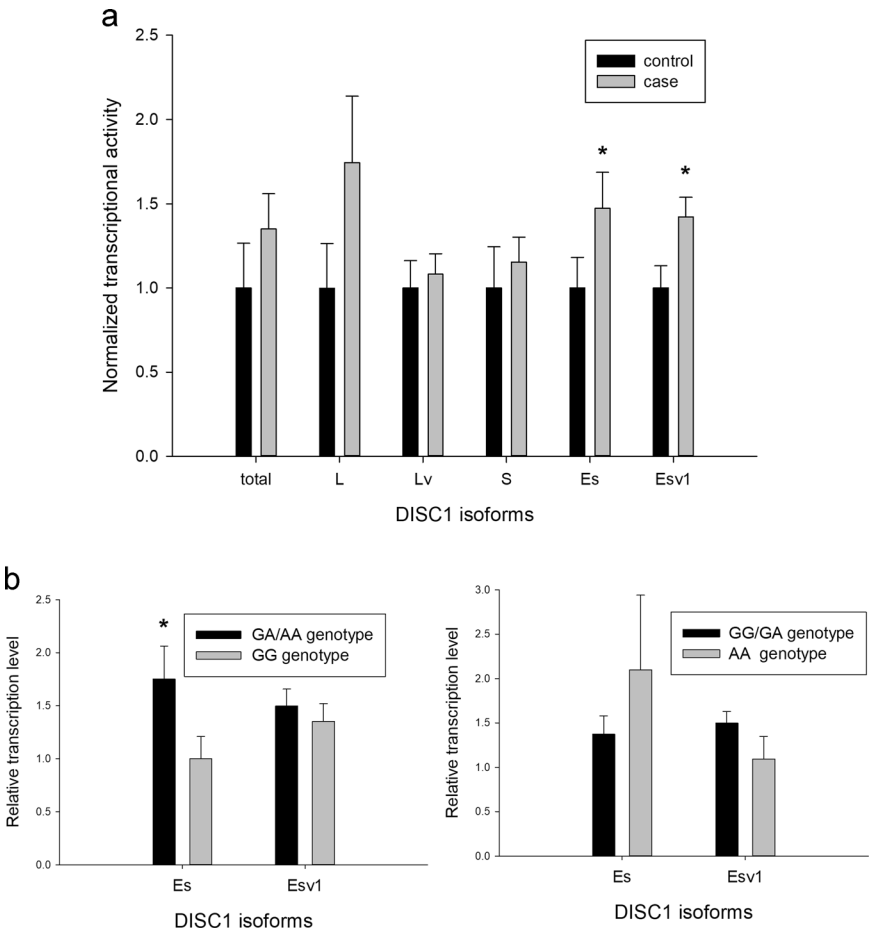

Fig. 1 Gene expression study of different *DISC1* isoforms in the EBV-transformed lymphoblastic cells of schizophrenia patients and controls. **a** Relative transcriptional levels of the different *DISC1* isoforms in the EBV-transformed lymphoblastic cells of schizophrenia patients ($n = 85$) and controls ($n = 36$). Data are normalized by the means of the control group and represented as mean ± SE. An asterisk denotes a significant difference ($p < 0.05$). **b** The comparisons between the genotypes of rs11122324 and the relative transcriptional levels of Es and Esv1 isoform. Data are represented as mean ± SE. An asterisk denotes a significant difference ($p < 0.05$). The level of Es isoform in patients with the AA ($n = 12$) and GA ($n = 43$) genotype is significantly higher than that in patients with the GG genotype ($n = 24$) using independent t-test. The level of Es isoform in patients with the AA genotype ($n = 12$) is not significantly different from that in patients with the GG ($n = 43$) and GA genotype ($n = 24$) using Mann–Whitney U-test. The comparisons of the levels of Esv1 isoform between patients with different genotypes are not significant

expression of other isoforms. The expression of Esv1 isoform was significantly higher in the CPT deficit subgroup (z-scores of undegraded CPT or degraded CPT < -2.5 as the same definition in the genetic association study) than that in the CPT non-deficit subgroup and control group (Supplementary Table 4). There were no significant differences in the expression of other isoforms among the three groups.

Luciferase reporter assay

The in-vitro functional study of rs11122324 using luciferase reporter assay revealed that the relative transcription activity (ratio to the control vector (pCMV4.10)) of the A-allele vector (pCMV4.10-DISC1-A) was significantly higher than that of the G-allele vector (pCMV4.10-DISC1-G), with an average increase of 20%. The findings were consistent and reproducible in the two cell lines ($t = 2.682$, d.f. $= 5$, $p = 0.044$ for U251 cell line; $t = 3.390$, d.f. $= 3$, $p = 0.043$ for HEK293T cell line) (Fig. 2).

Discussion

First, the distribution of genotypes of two SNPs, rs11122324 (at 3′-UTR of Es and Esv1 isoform of *DISC1*) and rs2793091 (at intron 4), was significantly different between the multiplex schizophrenia patients with deficient sustained attention and the normal controls. The proportions of AA genotypes of the two SNPs were significantly higher in the patient subgroup. The patients with AA genotypes of the two SNPs had poorer CPT performance. Second, the mRNA expressions of Es and Esv1 isoforms were significantly higher in the EBV-transformed lymphoblastic cells of schizophrenia patients than those of controls. The patients with A-allele of rs11122324 had higher expression of Es isoform than those without A-allele. The expression level of Esv1 isoform was negatively correlated to the performance of CPT. Lastly, the vector with A-allele of rs11122324 in 3′-UTR of DISC1 had significantly higher relative luciferase activity than that with G-allele.

Although significant association was not found between *DISC1* and schizophrenia as a whole, two SNPs of *DISC1* were found to be associated with a specific subgroup of schizophrenia, i.e., multiplex schizophrenia patients with deficient sustained attention. Two-SNP haplotypes composed of rs2793092 (at intron 3) and rs2793091 (at intron 4) were found to have significant transmission distortion, especially in the multiplex schizophrenia families with deficient sustained attention in our previous study [10]. After excluding the overlapping multiplex sample (87 multiplex patients) used in the previous study, the association results remained similar (Supplementary Table 5).

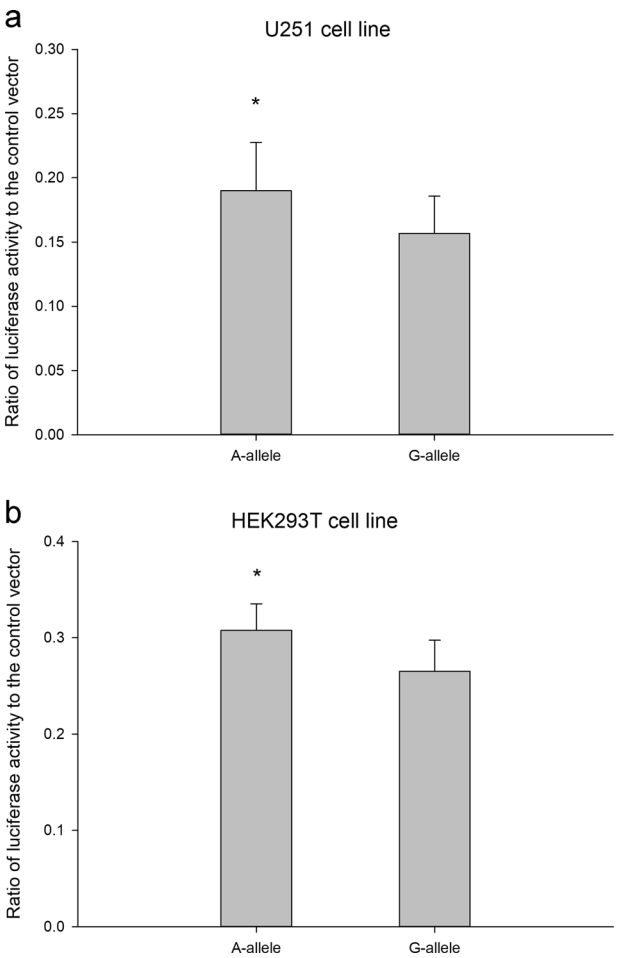

Fig. 2 Luciferase reporter assays for different alleles of rs11122324. **a** Transfection experiments in U251 cell line. Ratio of luciferase activity compared with the control vector (pCMV4.10) in the two vectors with different alleles of rs11122324 of DISC1 3′-UTR (pCMV4.10-DISC1-A and pCMV4.10-DISC1-G). Data are represented as mean ± SE. An asterisk denotes a significant difference ($p < 0.05$). **b** Transfection experiments in HEK293T cell line. Data are represented as mean ± SE. An asterisk denotes a significant difference ($p < 0.05$)

Therefore, we independently replicated the association evidence of *DISC1* with multiplex schizophrenia patients with deficient sustained attention and the association region was across intron 3 and intron 4.

The two SNPs are not in the same haplotype block according to Haploview [40]. Therefore, the association signals brought up by the two SNPs are likely to be independent. The SNP rs11122324 is located at the 3′-UTR of Es and Esv1 isoforms. The AA genotype was over-represented in multiplex patients with deficient attention. The A-allele correlated with higher mRNA expression of Es isoform in patients and in-vitro function study showed that the A-allele was associated with higher expression activity than the G-allele. Higher expression of Es and Esv1 isoforms were found in schizophrenia patients. All of these consistent findings imply that rs11122324 may have

functional influences on the mRNA expression of Es or Esv1 isoform, which contributes to the disease mechanism in the subgroup of multiplex schizophrenia with impaired sustained attention. The genotypes of SNP rs11122324 were reported to be significantly associated with the mRNA expression of total *DISC1* in the lymphocytes of healthy individuals and the neighboring SNP rs2487453 (about 4 kb distal from rs11122324) was significantly associated with the thickness of cingulate cortex in healthy individuals [15].

The SNP rs2793091 was also reported to be significantly associated with multiplex schizophrenia families with deficient sustained attention in our previous study [10]. However, the functional relevance of this SNP is still unknown. Clarification is needed about whether the SNP itself is a risk polymorphism or is in linkage disequilibrium with other risk variants.

Another possibility for the association signals brought up by the two SNPs might arise from the functional (rare or low-frequency) variants near the two SNPs. Multiplex schizophrenia patients come from families with heavier genetic loading than simplex schizophrenia patients and have been reported to have more severe impairments in some neurocognitive functions, including sustained attention [41]. Previous studies also suggest that familial schizophrenia (with higher genetic loadings) is the more serious subtype with more minor physical anomalies, more cognitive impairment, and severe structural brain abnormalities compared with sporadic schizophrenia (with lower genetic loadings) [42, 43]. Patients from multiplex families may have more inherited private rare or low-frequency variants than those from simplex families. In the present study, the AA genotypes of the two SNPs were found to be more prevalent in the multiplex patients with deficient sustained attention. If a significant proportion of the patient subgroup with AA genotype have nearby private rare or low-frequency variants, which are inherited in the multiplex families and have more functional impacts on sustained attention, it may be consistent with the association pattern observed in this study, which showed significance only for genotype distribution, but not for allele frequency. Three rare or low-frequency variants were identified within 735 bp proximal to rs11122324 by direct sequencing, two of which are not identified in our controls and have much higher allele frequency compared with those in the public resources (Supplementary Table 2). Rare or low-frequency variants in *DISC1* may have roles in the specific subgroup of familial schizophrenia, although we found associations with two common variants.

The original evidence of *DISC1* association came from a rare chromosome abnormality in a big Scottish family [4]. Song et al. [44] found 5 ultra-rare missense variants in 6 schizophrenia patients, which were not found in the 288 controls and also were absent in a pool of 10,000 control alleles. The burden analysis was significant. An increased burden of rare missense variants were also reported in exon 11 of *DISC1* for schizoaffective disorder [45]. A deep sequencing of 528 kb of *DISC1* genomic region in 653 psychiatric patients and 889 controls found 2010 rare variants, 62% of which are not found in 1000 Genomes, and the investigators concluded that many *DISC1* SNPs remained undiscovered and are essentially private [46]. Although we sequenced only 24 kb of *DISC1* genomic region in only 100 schizophrenia patients, 19% of the identified variants were not found in 1000 Genomes and other public variation resources, which is consistent with the conclusions of that study [46]. However, our sequencing range and sample size were too small to make any conclusion about the roles that rare variants of *DISC1* might play in schizophrenia.

We found the association evidence between the two SNPs of DISC1 and the sustained attention of schizophrenia. However, because of the lack of CPT data in the control group in this genetic study, we cannot test the genetic association between DISC1 and the dimension of sustained attention in general. We found significant association between the expression level of Esv1 isoform and sustained attention in both schizophrenia and normal control. Although DISC1 may not be a key risk factor for schizophrenia nor for other disorders that are defined by the DSM diagnostic system, our study suggests that it may confer a genetic risk at the level of sustained attention that underlies several major mental disorders.

In the present study, the mRNA levels of Es and Esv1 isoforms were found to be significantly higher in the lymphoblastic cells of schizophrenia patients. A previous postmortem brain study reported a significant increase of mRNA expression of Esv1 isoform in the hippocampus of schizophrenia patients [27]. The function of the Es/Esv1 isoforms is still unknown, although enriched expression was reported in the fetal brain [27]. The functional relevance of increased level of Es and Esv1 isoform may be associated with schizophrenia in a dominant-negative manner, because the truncated proteins translated by the ultrashort isoforms lack the C terminus predicted to mediate protein–protein interactions with a large number of proteins. Transfection of C-terminally truncated DISC1 into cells leads to aberrant phenotypes, such as disruption of interactions with other proteins, including NDEL1 [47], MAP1A, MIPT3, ATF4/5 [48], and PDE4B [23], aberrant mitochondrial function [49], and inhibition of neurite outgrowth [20, 47]. However, the definite effects of overexpression of these ultrashort isoforms for cells and animals still remain to be clarified.

This study has several limitations. First, the association evidence of the two SNPs was limited to the genotype distribution, but not allele frequency. Second, although patients with the A-allele had higher expression of Es

isoform, the association between genotype and expression is not compatible with the recessive model in the genetic association. Third, the higher expression of mRNAs of Es and Esv1 isoform between patients and controls is not significant after multiple correction.

Findings of this study suggest that the SNP rs11122324 at the 3′-UTR of Es and Esv1 isoforms may be a true risk polymorphism through its influence on the expression of extra-short isoforms. Alternatively, some true functional variants may be harbored near the two SNPs in the specific familial schizophrenia subgroup. Deep sequencing for the specific subgroup is worth performing. *DISC1* may also play roles on the neural mechanism of sustained attention in schizophrenia. The functional impact of elevated extra-short isoforms of DISC1 upon cell and animal models is also worth further exploration.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Lichtenstein P, Yip BH, Bjork C, Pawitan Y, Cannon TD, Sullivan PF, et al. Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet. 2009;373:234–9.
2. Hwu HG, Liu CM, Fann CS, Ou-Yang WC, Lee SF. Linkage of schizophrenia with chromosome 1q loci in Taiwanese families. Mol Psychiatry. 2003;8:445–52.
3. Ekelund J, Hovatta I, Parker A, Paunio T, Varilo T, Martin R, et al. Chromosome 1 loci in Finnish schizophrenia families. Hum Mol Genet. 2001;10:1611–7.
4. Millar JK, Christie S, Anderson S, Lawson D, Hsiao-Wei Loh D, Devon RS, et al. Genomic structure and localisation within a linkage hotspot of Disrupted In Schizophrenia 1, a gene disrupted by a translocation segregating with schizophrenia. Mol Psychiatry. 2001;6:173–8.
5. Chubb JE, Bradshaw NJ, Soares DC, Porteous DJ, Millar JK. The DISC locus in psychiatric illness. Mol Psychiatry. 2008;13:36–64.
6. Mathieson I, Munafo MR, Flint J. Meta-analysis indicates that common variants at the DISC1 locus are not associated with schizophrenia. Mol Psychiatry. 2012;17:634–41.
7. Kinoshita M, Numata S, Tajima A, Ohi K, Hashimoto R, Shimodera S, et al. Meta-analysis of association studies between DISC1 missense variants and schizophrenia in the Japanese population. Schizophr Res. 2012;141:271–3.
8. Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. Nature. 2014;511:421–7.
9. Wang HY, Liu Y, Yan JW, Hu XL, Zhu DM, Xu XT, et al. Gene polymorphisms of DISC1 is associated with schizophrenia: evidence from a meta-analysis. Prog Neuropsychopharmacol Biol Psychiatry. 2018;81:64–73.
10. Liu YL, Fann CS, Liu CM, Chen WJ, Wu JY, Hung SI, et al. A single nucleotide polymorphism fine mapping study of chromosome 1q42.1 reveals the vulnerability genes for schizophrenia, GNPAT and DISC1: association with impairment of sustained attention. Biol Psychiatry. 2006;60:554–62.
11. Hennah W, Tuulio-Henriksson A, Paunio T, Ekelund J, Varilo T, Partonen T, et al. A haplotype within the DISC1 gene is associated with visual memory functions in families with a high density of schizophrenia. Mol Psychiatry. 2005;10:1097–103.
12. Cannon TD, Hennah W, van Erp TG, Thompson PM, Lonnqvist J, Huttunen M, et al. Association of DISC1/TRAX haplotypes with schizophrenia, reduced prefrontal gray matter, and impaired short- and long-term memory. Arch Gen Psychiatry. 2005;62:1205–13.
13. Shaikh M, Hall MH, Schulze K, Dutt A, Li K, Williams I, et al. Effect of DISC1 on the P300 waveform in psychosis. Schizophr Bull. 2011;39:161–7.
14. Callicott JH, Straub RE, Pezawas L, Egan MF, Mattay VS, Hariri AR, et al. Variation in DISC1 affects hippocampal structure and function and increases risk for schizophrenia. Proc Natl Acad Sci USA. 2005;102:8627–32.
15. Carless MA, Glahn DC, Johnson MP, Curran JE, Bozaoglu K, Dyer TD, et al. Impact of DISC1 variation on neuroanatomical and neurocognitive phenotypes. Mol Psychiatry. 2011;16:1063.
16. Sprooten E, Sussmann JE, Moorhead TW, Whalley HC, Ffrench-Constant C, Blumberg HP, et al. Association of white matter integrity with genetic variation in an exonic DISC1 SNP. Mol Psychiatry. 2011;16:688–9.
17. Brandon NJ, Sawa A. Linking neurodevelopmental and synaptic theories of mental illness through DISC1. Nat Rev Neurosci. 2011;12:707–22.
18. Ishizuka K, Kamiya A, Oh EC, Kanki H, Seshadri S, Robinson JF, et al. DISC1-dependent switch from progenitor proliferation to migration in the developing cortex. Nature. 2011;473:92–96.
19. Kubo K, Tomita K, Uto A, Kuroda K, Seshadri S, Cohen J, et al. Migration defects by DISC1 knockdown in C57BL/6, 129X1/SvJ, and ICR strains via in utero gene transfer and virus-mediated RNAi. Biochem Biophys Res Commun. 2010;400:631–7.
20. Ozeki Y, Tomoda T, Kleiderlein J, Kamiya A, Bord L, Fujii K, et al. Disrupted-in-Schizophrenia-1 (DISC-1): mutant truncation

prevents binding to NudE-like (NUDEL) and inhibits neurite outgrowth. Proc Natl Acad Sci USA. 2003;100:289–94.
21. Tsuboi D, Kuroda K, Tanaka M, Namba T, Iizuka Y, Taya S, et al. Disrupted-in-schizophrenia 1 regulates transport of ITPR1 mRNA for synaptic plasticity. Nat Neurosci. 2015;18:698–707.
22. Seshadri S, Faust T, Ishizuka K, Delevich K, Chung Y, Kim SH, et al. Interneuronal DISC1 regulates NRG1-ErbB4 signalling and excitatory-inhibitory synapse formation in the mature cortex. Nat Commun. 2015;6:10118.
23. Millar JK, Pickard BS, Mackie S, James R, Christie S, Buchanan SR, et al. DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate cAMP signaling. Science. 2005;310:1187–91.
24. Niwa M, Cash-Padgett T, Kubo KI, Saito A, Ishii K, Sumitomo A, et al. DISC1 a key molecular lead in psychiatry and neurodevelopment: no-more disrupted-in-schizophrenia 1. Mol Psychiatry. 2016;21:1488–9.
25. Teng S, Thomson PA, McCarthy S, Kramer M, Muller S, Lihm J, et al. Rare disruptive variants in the DISC1 Interactome and Regulome: association with cognitive ability and schizophrenia. Mol Psychiatry. 2018;23:1270–7.
26. Tomoda T, Sumitomo A, Jaaro-Peled H, Sawa A. Utility and validity of DISC1 mouse models in biological psychiatry. Neuroscience. 2016;321:99–107.
27. Nakata K, Lipska BK, Hyde TM, Ye T, Newburn EN, Morita Y, et al. DISC1 splice variants are upregulated in schizophrenia and associated with risk polymorphisms. Proc Natl Acad Sci USA. 2009;106:15873–8.
28. Rastogi A, Zai C, Likhodi O, Kennedy JL, Wong AH. Genetic association and post-mortem brain mRNA analysis of DISC1 and related genes in schizophrenia. Schizophr Res. 2009;114:39–49.
29. Kumarasinghe N, Beveridge NJ, Gardiner E, Scott RJ, Yasawardene S, Perera A, et al. Gene expression profiling in treatment-naive schizophrenia patients identifies abnormalities in biological pathways involving AKT1 that are corrected by antipsychotic medication. Int J Neuropsychopharmacol. 2013;16:1483–503.
30. Rampino A, Walker RM, Torrance HS, Anderson SM, Fazio L, Di Giorgio A, et al. Expression of DISC1-interactome members correlates with cognitive phenotypes related to schizophrenia. PLoS ONE. 2014;9:e99892.
31. Dahoun T, Trossbach SV, Brandon NJ, Korth C, Howes OD. The impact of Disrupted-in-Schizophrenia 1 (DISC1) on the dopaminergic system: a systematic review. Transl Psychiatry. 2017;7:e1015.
32. Hwu HG, Chen CH, Hwang TJ, Liu CM, Cheng JJ, Lin SK, et al. Symptom patterns and subgrouping of schizophrenic patients: significance of negative symptoms assessed on admission. Schizophr Res. 2002;56:105–19.
33. Hwu HG, Faraone SV, Liu CM, Chen WJ, Liu SK, Shieh MH, et al. Taiwan schizophrenia linkage study: the field study. Am J Med Genet B Neuropsychiatr Genet. 2005;134:30–36.
34. Hwu HG Psychiatric diagnostic assessment. 2nd ed. Taipei: Publication Committee, College of Medicine, National Taiwan University; 1999.
35. Chen WJ, Hsiao CK, Hsiao LL, Hwu HG. Performance of the continuous performance test among community samples. Schizophr Bull. 1998;24:163–74.
36. Chang SS, Liu CM, Lin SH, Hwu HG, Hwang TJ, Liu SK, et al. Impaired flush response to niacin skin patch among schizophrenia patients and their nonpsychotic relatives: the effect of genetic loading. Schizophr Bull. 2009;35:213–21.
37. Pan WH, Fann CS, Wu JY, Hung YT, Ho MS, Tai TH, et al. Han Chinese cell and genome bank in Taiwan: purpose, design and ethical considerations. Hum Hered. 2006;61:27–30.
38. Fu CL & Yang UC. ISVdb: Integrated splicing variants database. National Yang-Ming University, Taipei, Taiwan; 2004.
39. Chen WJ, Chang CH, Liu SK, Hwang TJ, Hwu HG. Sustained attention deficits in nonpsychotic relatives of schizophrenic patients: a recurrence risk ratio analysis. Biol Psychiatry. 2004;55:995–1000.
40. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005;21:263–5.
41. Walker E, Shaye J. Familial schizophrenia. A predictor of neuromotor and attentional abnormalities in schizophrenia. Arch Gen Psychiatry. 1982;39:1153–6.
42. Sautter FJ, McDermott BE, Cornwell J, Black FW, Borges A, Johnson J, et al. Patterns of neuropsychological deficit in cases of schizophrenia spectrum disorder with and without a family history of psychosis. Psychiatry Res. 1994;54:37–49.
43. Lui S, Deng W, Huang X, Jiang L, Ouyang L, Borgwardt SJ, et al. Neuroanatomical differences between familial and sporadic schizophrenia and their parents: an optimized voxel-based morphometry study. Psychiatry Res. 2009;171:71–81.
44. Song W, Li W, Feng J, Heston LL, Scaringe WA, Sommer SS. Identification of high risk DISC1 structural variants with a 2% attributable risk for schizophrenia. Biochem Biophys Res Commun. 2008;367:700–6.
45. Green EK, Grozeva D, Sims R, Raybould R, Forty L, Gordon-Smith K, et al. DISC1 exon 11 rare variants found more commonly in schizoaffective spectrum cases than controls. Am J Med Genet B Neuropsychiatr Genet. 2011;156B:490–2.
46. Thomson PA, Parla JS, McRae AF, Kramer M, Ramakrishnan K, Yao J, et al. 708 Common and 2010 rare DISC1 locus variants identified in 1542 subjects: analysis for association with psychiatric disorder and cognitive traits. Mol Psychiatry. 2014;19:668–75.
47. Kamiya A, Tomoda T, Chang J, Takaki M, Zhan C, Morita M, et al. DISC1-NDEL1/NUDEL protein interaction, an essential component for neurite outgrowth, is modulated by genetic variations of DISC1. Hum Mol Genet. 2006;15:3313–23.
48. Morris JA, Kandpal G, Ma L, Austin CP. DISC1 (Disrupted-In-Schizophrenia 1) is a centrosome-associated protein that interacts with MAP1A, MIPT3, ATF4/5 and NUDEL: regulation and loss of interaction with mutation. Hum Mol Genet. 2003;12:1591–608.
49. Millar JK, James R, Christie S, Porteous DJ. Disrupted in schizophrenia 1 (DISC1): subcellular targeting and induction of ring mitochondria. Mol Cell Neurosci. 2005;30:477–84.