Journal of Affective Disorders 166 (2014) 103–107

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Brief report

No evidence of *DISC1*-associated morphological changes in the hippocampus, anterior cingulate cortex, or striatum in major depressive disorder cases and healthy controls

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ARTICLE INFO

Article history:
Received 20 February 2014
Received in revised form
30 April 2014
Accepted 1 May 2014
Available online 11 May 2014

Keywords:
DISC1
Major depressive disorder
Imaging genetics
Hippocampus

ABSTRACT

Background: *DISC1* imaging genetics studies in healthy controls, schizophrenia, and bipolar disorder cases have revealed morphological changes in brain regions involved in the pathophysiology of psychiatric disease including the hippocampus, anterior cingulate cortex (ACC), and the striatum. However, many of these studies have yielded discordant findings so there is a need for replication. Furthermore, despite evidence from human genetic studies and animal models implicating *DISC1* in major depressive disorder (MDD), a *DISC1* imaging genetics study in MDD cases has yet to be published. Thus, using neuroimaging data from MDD cases and a large sample of healthy controls we aimed to identify morphological changes representing neurobiological mechanisms underlying the association between *DISC1* and MDD.

Methods: We utilized structural magnetic resonance imaging (sMRI) data from 512 healthy controls and 171 current MDD (SCID interview) cases, each with genotype data for non-synonymous *DISC1* SNPs rs3738401, rs6675281, and rs821616.

Results: Region of interest analyses failed to reveal *DISC1*-associated morphological changes in the hippocampus, ACC, or striatum in MDD patients and healthy controls. Whole brain exploratory analyses identified a nominally significant cluster mapping to the border of the precentral and postcentral gyri associated with rs821616 in healthy controls only (p(uncorrected) < 0.001).

Limitations: We focused our analyses exclusively on three, but previously heavily studied, SNPs in *DISC1*.

Conclusions: Our findings suggest that morphological changes in the hippocampus, ACC, and/or striatum of MDD patients do not represent neurobiological mechanisms underlying the association between *DISC1* and MDD. However, we urge replication in independent samples of MDD cases.

1. Introduction

Disrupted in schizophrenia candidate 1 (*DISC1*) was initially identified as a candidate gene for psychiatric disease following the discovery of a balanced chromosomal translocation (t(1; 11)(q42.1; q14.3)) disrupting *DISC1* that has co-segregated with schizophrenia (SZ), bipolar disorder (BD), and major depressive disorder (MDD) in a large Scottish pedigree (Blackwood et al., 2001; Millar et al., 2000). Although *DISC1* has primarily received attention within the context of SZ, there is now mounting evidence from human genetic association studies and animal models indicating a role for *DISC1* in the etiology of affective disorders including BD and MDD (Hashimoto et al., 2006; Hodgkinson et al., 2004; Jaaro-Peled et al., 2013; Schosser et al., 2010; Thomson et al., 2013).

The precise molecular and cellular functioning of *DISC1* has yet to be clearly defined, though evidence indicates *DISC1* to be involved in processes regulating neuronal development including proliferation, differentiation, migration, and neurite outgrowth (Wu et al., 2013). Indeed, in keeping with the above functional themes, a large body of evidence from human imaging genetics studies indicates a potential role for *DISC1* in the regulation of brain morphology (Duff et al., 2013). For example, non-synonymous SNPs (i.e., rs821616, Ser704Cys; rs6675281, Leu607Phe) in *DISC1* have been associated with differences in

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http://dx.doi.org/10.1016/j.jad.2014.05.001
0165-0327/

hippocampal gray matter ($n=34$ and 86) (Callicott et al., 2005; Di Giorgio et al., 2008) and striatal ($n=54$) (Chakravarty et al., 2012) volumes in healthy controls, as well as anterior cingulate cortex (ACC) volume in healthy developing children/adolescents ($n=40$), healthy adults ($n=19$), and SZ cases ($n=14$) (Raznahan et al., 2011; Szeszko et al., 2008).

However, there have been inconsistencies amongst these findings (i.e., Callicott et al., 2005; Di Giorgio et al., 2008) and so replication in independent samples of healthy controls is required. Furthermore, although the hippocampus, ACC, and striatum are believed to be key structures in the pathophysiology of MDD, to our knowledge an article reporting on *DISC1*-associated morphology in MDD cases (either positive or negative) has yet to be published. Thus, here we have performed a *DISC1* imaging genetics study in large samples of MDD cases ($n=171$) and healthy controls ($n=512$) consisting of region-of-interest (ROI) analyses focusing on the hippocampus, ACC, and striatum, as well as exploratory whole brain analyses, to determine whether *DISC1* polymorphisms are associated with morphological changes. Findings from these analyses are expected to provide information concerning the potential neurobiological mechanisms driving the apparent relationship between *DISC1* and MDD (Hashimoto et al., 2006; Jaaro-Peled et al., 2013; Schosser et al., 2010; Thomson et al., 2013).

2. Methods and materials

2.1. Sample characteristics

Two independent samples of central European ancestry have been collected at the University of Münster. Sample 1 consisted of $n=512$ healthy subjects (see Table 1 for demographic and clinical characteristics) after standard imaging quality assurance procedures (Baune et al., 2012a, 2012b). All subjects were thoroughly investigated by experienced psychologists and were free from any life-time history of psychiatric disorders according to DSM-IV criteria (American Psychiatric Association, 1994), as diagnosed with the SCID interview (Wittchen et al., 1997). Furthermore, any psychotropic medication was an exclusion criterion.

Sample 2 consisted of $n=171$ patients (Table 1) suffering from major depressive disorder (MDD) having a current depressive episode, as diagnosed with the SCID interview (Wittchen et al., 1997). Comorbid diagnoses of substance related disorders or psychotic disorders were exclusion criteria. All patients were under inpatient treatment at the University Hospital of Münster at the time of testing and most patients were medicated. According to chi²-tests, there were no differences for any genotype regarding presence/absence of antidepressant groups (SSRI, SNRI, Tricyclics, MAO-I, atypical) ($p>0.12$).

Common exclusion criteria for both samples were any neurological abnormalities including stroke, epilepsy, dementia, or head trauma, and the usual MRI-contraindications. The study was approved by the Ethics Committee of the University of Münster. After complete description of the study to the participants, written informed consent was obtained.

2.2. DNA extraction and genotyping

DNA was extracted from whole blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN, VIC, Australia). Four *DISC1* SNPs were selected for genotyping on the basis that (i) they have featured heavily in *DISC1* imaging genetics studies to date (Duff et al., 2013) and (ii) they are all non-synonymous coding. All four SNPs were genotyped according to published protocols using the iPLEX™ multiplex genotyping assay on the Sequenom MassARRAY platform (Oeth et al., 2009).

2.3. Structural MRI acquisition and morphometry methods

MRI methods and statistical approaches have been published recently (Baune et al., 2012a, 2012b; Dannlowski et al., 2012, in press). Briefly, T1 weighted high resolution images were acquired on a 3T-MRI scanner (Gyroscan Intera 3T, Philips Medical Systems, Best, NL) with a 3D 'Turbo Field Echo' sequence, TR=7.4 ms, TE=3.4 ms, FA=9°, 2 signal averages, inversion prepulse every 814.5 ms, acquired over a field of view of 256 mm (FH) × 204 mm (AP) × 160 mm (RL, nominal slice selection direction) with 1 mm resolution in all directions, frequency encoding in FH direction, phase encoding in AP and RL direction, reconstructed to cubic voxels of 0.5 mm × 0.5 mm × 0.5 mm.

The VBM8-toolbox (http://dbm.neuro.uni-jena.de/vbm) was used for pre-processing the structural images with default parameters in both samples. Images were bias-corrected, tissue classified, and normalized to MNI-space using linear (12-parameter affine) and non-linear transformations, within a unified model (Ashburner and Friston, 2005) including high-dimensional DARTEL-normalization. Gray matter segments were modulated only by the non-linear components in order to preserve actual gray matter values locally (modulated GM volumes). Using these procedures, no further correction for total brain volume is required anymore. The modulated gray matter images were smoothed with a Gaussian kernel of 8 mm FWHW.

Group statistics were calculated using SPM8 within each sample separately. For each *DISC1* SNP, one-factorial ANOVAs were conducted using genotype group as between-subjects factors, using both a three group genotype model and a two group model (i.e., grouping homozygous subjects for the minor allele together with heterozygotes), unless a minimum cell size of $N=20$ subjects/group was obtained. Unequal variance was assumed for modeling, and age and gender were added to all models as nuisance regressors. An absolute masking threshold of 0.1 was used.

Table 1
Demographic and clinical characteristics of study samples.

	MDD cases ($n=171$)	Healthy controls ($n=512$)	Statistics
Age	38.6 ± 11.7	33.3 ± 11.3	$t_{(681)}=5.16$, $p<0.001$
Sex	66M, 105F	223M, 289F	$\chi^2_{(1)}=1.29$, $p=0.26$
Years in education	14.2 ± 2.2	14.8 ± 2.1	$t_{(681)}=3.25$, $p=0.001$
Verbal IQ (MWT) (Lehrl, 1995)	111.4 ± 13.4	116.0 ± 12.5	$t_{(594)}=3.84$, $p<0.001$
BDI (Beck and Steer, 1987)	23.3 ± 11.1	2.4 ± 3.1	$t_{(662)}=37.9$, $p<0.001$
STAI (Laux et al., 1981)	58.8 ± 9.3	32.2 ± 6.9	$t_{(615)}=37.0$, $p<0.001$

M: male; F: female; MWT: Mehrfachwahl-Wortschatz-Intelligenz test (multiple choice vocabulary intelligence test); BDI: Beck's Depression Inventory; STAI: State-Trait Anxiety Inventory. Age X SNP genotype one-way ANOVA p-values; rs3738401: $p=0.63$ (cases), $p=0.28$ (controls); rs6675281: $p=0.045$ (cases), $p=0.80$ (controls); rs821686: $p=0.73$ (cases), $p=0.36$ (controls). χ^2 p-Values for sex distribution across each SNP; rs3738401: $p=0.66$ (cases), $p=0.99$ (controls); rs6675281: $p=0.19$ (cases), $p=0.46$ (controls); rs821686: $p=0.36$ (cases), $p=0.48$ (controls).