Psychiatry Research: Neuroimaging 224 (2014) 311–318

Contents lists available at ScienceDirect

Psychiatry Research: Neuroimaging

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

Cognitive and structural neuroimaging characteristics of schizophrenia patients with large, rare copy number deletions

Andrew Kenneth Martin [a,*], Gail Robinson [b], David Reutens [c], Bryan Mowry [a,d]

[a] University of Queensland, Queensland Brain Institute, St Lucia Queensland 4072, Australia
[b] University of Queensland, School of Psychology, St Lucia Queensland 4072, Australia
[c] University of Queensland, Centre for Advanced Imaging, St Lucia Queensland 4072, Australia
[d] University of Queensland, Queensland Centre for Mental Health Research, Wacol 4076, Queensland

ARTICLE INFO

Article history:
Received 13 April 2014
Received in revised form
11 August 2014
Accepted 7 October 2014
Available online 18 October 2014

Keywords:
Schizophrenia
Cognition
Deletions
Copy number variants
VBM
DTI

ABSTRACT

Large (> 500 Kb), rare (frequency < 1%) deletions are associated with risk for schizophrenia. The aim of the study was to characterise patients with these deletions using measures of cognition, grey-matter volume and white-matter integrity. Patients with schizophrenia and large, rare deletions (SZ-del) ($n=17$) were assessed on a test of intelligence, the Wechsler Abbreviated Scale of Intelligence (WASI), and compared with age- and sex-matched schizophrenia patients without large, rare deletions (SZ-nodel) ($n=65$), and healthy controls (HCs) ($n=50$). Regional grey-matter differences were investigated using voxel-based morphometry (SZ-del=9; SZ-nodel=26; HC=19). White-matter integrity was assessed using fractional anisotropy (SZ-del=9; SZ-nodel=24; HC=15). Compared with schizophrenia patients without large, rare deletions, those with large, rare deletions had lower IQ; greater grey-matter volume in clusters with peaks in the left and right cerebellum, left hippocampus, and right rectal gyrus; and increased white-matter anisotropy in the body and genu of the corpus callosum. Compared with healthy controls, patients with large, rare deletions had reduced grey matter volume in the right calcarine gyrus. In sum, patients with large, rare deletions had structural profiles intermediate to those observed in healthy controls and schizophrenia patients without large, rare deletions, but had greater impairment in intelligence.

1. Introduction

Schizophrenia is a highly heritable disorder with genetic factors explaining approximately 80% of the variance (Cardno and Gottesman, 2000). The emerging picture is one of polygenic risk factors, including structural variants such as copy number variants (CNVs) (for review, see Mowry and Gratten (2013)). Specific CNVs, such as deletions at 22q11.2 confer risk for schizophrenia and, to date, several genome-wide significant CNVs have been identified (International Schizophrenia Consortium, 2008; Stefansson et al., 2008; Levinson et al., 2011; Szatkiewicz et al., 2014). Further to this, large (> 500 Kb), rare (frequency < 1%) copy number deletions in general have been identified in a disproportionate number of cases compared with controls (Szatkiewicz et al., 2014), suggesting a role in schizophrenia risk. Although smaller and more common CNVs are almost certainly going to be discovered and found to be important in understanding the role of structural variation in schizophrenia, large, rare deletions in general appear robustly associated with risk and are worthy of examination at the phenotypic level.

Cognitive impairment is a core feature of the disorder, with decline often occurring before the onset of illness (Reichenberg et al., 2010). Another robust finding is reduction in brain grey matter, specifically in fronto-temporal regions (Vita et al., 2012) and reduced regional white-matter integrity (Samartzis et al., 2014) in patients with schizophrenia. Recently, there have been a number of studies aiming to characterise the effects of CNVs, especially deletions, on both patients with schizophrenia and healthy controls. Yeo et al. (2013) found a negative correlation between general cognitive ability and deletion burden as measured by number of deletions across the genome, which included all deletions regardless of size and defined rare as occurring in less than 3% of their sample. They also identified a positive correlation between ventricular size and deletion burden. As this relationship was not identified in a comparable group of healthy controls, the authors propose that deletions cause greater neurodevelopmental instability and a greater susceptibility for other genetic or environmental factors to direct development towards schizophrenia. Another study found no relationship between deletion or duplication burden and total brain, white-matter, and grey-matter volume in 173 schizophrenia patients and 176 healthy controls (Terwisscha van Scheltinga et al., 2012), and the same group provided evidence that intelligence is not associated with CNVs or

* Corresponding author. Tel.: +61 7 3346 6351.
E-mail address: a.martin11@uq.edu.au (A. Kenneth Martin).

http://dx.doi.org/10.1016/j.pscychresns.2014.10.006
0925-4927/

polygenic risk scores (van Scheltinga et al., 2013). Importantly, both this study and the Yeo et al. study grouped CNVs across the genome and analyses were performed on "total burden" rather than CNVs associated with schizophrenia risk, as reported in the current study. In a study by Stefansson et al. (2014) healthy controls without schizophrenia, but who carry schizophrenia risk- associated CNVs, were assessed on a number of cognitive measures. Performance was found to be intermediate between that of healthy controls and patients with schizophrenia, suggesting that the CNVs confer an effect on cognitive functioning independent of disease status.

Although the effects of overall deletion burden may provide useful insight into the phenotypic role of mutations in schizophrenia, of most interest will be characterising the deletions that confer risk for disease. Large (> 500 Kb), rare (< 1% frequency) deletions are associated with increased risk for schizophrenia (Owen et al., 2010; Szatkiewicz et al., 2014), and characterising these patients may provide a better understanding of the role for large, rare deletions in schizophrenia risk. Recently, large, rare deletions have been associated with reduced cannabis abuse/dependence and a later age at onset (Martin et al., 2014a). In a sample drawn from this cohort, the current study will investigate IQ, grey-matter, and white-matter differences between healthy controls and patients with schizophrenia with and without large, rare deletions. It is hypothesised that patients with large, rare deletions will show greater general cognitive impairment as measured using an estimate of IQ. In addition, patients with and without large, rare deletions will have regional structural differences as measured by voxel-based morphometry (VBM) for grey-matter volume and fractional anisotropy (FA) for white-matter integrity.

2. Methods

2.1. Participants

All schizophrenia patients were recruited from the Australian subsample of a genome-wide association study (Levinson et al., 2011). Individuals were comprehensively ascertained by trained clinicians using the following measures: (i) the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al., 1994); (ii) Family Interview for Genetic Studies (FIGS) (Gershon et al., 1988; Maxwell, 1992); (iii) information extracted from all available medical records; (iv) narrative summary prepared by the interviewer and based on all information obtained from the DIGS, FIGS and medical records. The narrative summary was invaluable in recording the first-hand impressions of the interviewer. This facilitated diagnostic assessment by augmenting the DIGS information, especially when the participant's responses lacked clarity; (v) Best Estimate Final Diagnosis (BEFD) (Leckman et al., 1982) was assigned by two experienced research psychiatrists who independently reviewed all available information and then conferred before assigning a consensus diagnosis; one of us (B.M.) reviewed every Australian case. Diagnostic inter-rater reliability was assessed using standard procedures (Suarez et al., 2006).

Of the 633 Australian cases, 60 were identified as having a large (> 500 Kb), rare (< 1% frequency) deletion. Seventeen were assessed on a measure of intelligence. Loss to follow-up occurred due to original participants being deceased, unable to be contacted, unwilling to participate, or unable to participate. There were no differences in clinical symptoms or total copy number deletion burden in those cognitively assessed and those unable or unwilling to participate. Of the 17, nine had structural neuroimaging to assess grey- and white-matter volume. Loss to follow-up for neuroimaging was due to the participant being unwilling or unable to undergo neuroimaging for medical reasons. Schizophrenia patients without large, rare deletions (SZ-nodel) were selected from the same GWAS and were age- and sex-matched to the patients with large, rare deletions (SZ-del). Healthy controls (HCs) were recruited randomly using a private company through the Queensland Centre for Mental Health Research, University of Queensland, Australia, and were age- and sex-matched to the patient groups. A trained psychiatric nurse or research worker (AM), under the supervision of a clinical neuropsychologist (GR), carried out all cognitive assessments in the participant's place of residence or at the Queensland Centre for Mental Health, Brisbane, Queensland, Australia. All neuroimaging took place at the Centre for Advanced Imaging, University of Queensland, Brisbane, Australia. Data collection occurred between 2011 and 2014.

2.2. Copy number variant identification

2.2.1. Original MGS study

Quality control, identification and analytic methods have been described previously (Levinson et al., 2011). In brief, DNAs were assayed using Affymetrix 6.0 genotyping arrays, which included approximately 900,000 single-nucleotide polymorphisms (SNPs) and approximately 900,000 copy number probes. CNVs were detected with the Birdseye module of Birdsuite software package (Korn et al., 2008). Quality-control steps for CNV calls included the following: duplicate assays to develop narrow and broad call criteria, exclusion of calls involving telomeres and centromeres, immunoglobulin genes, and occurrence on one/two plates only. DNA samples were also subject to quality-control steps. Plots of "regions of interest" calls were visually inspected with confirmation by a second calling algorithm Quantitative polymerase chain reaction (qPCR) confirmed the presence of selected CNVs. PLINK (Purcell et al., 2007) point-wise analyses were conducted for all rare CNVs (with < 1% frequency) and those of more than 100,000 bp.

2.2.2. Australian MGS SCZ sub-set

Most MGS DNAs were extracted from Epstein–Barr virus transformed lymphoblastic cell lines, and because EB transformation can create CNVs (Wang et al., 2007), we sought fresh blood samples from Australian MGS participants and extracted DNA from whole blood for confirmation of the CNVs documented in MGS. A proportion of the CNVs were confirmed for the purposes of another study using TaqMan Copy Number assays (Applied Biosystems) following recommended protocols on a StepOnePlus real-time PCR instrument (Applied Biosystems). Target assays were run simultaneously with reference assays which detect sequences that are known to have two copies in viable diploid human cells. Copy number for the targets was determined using the comparative C_T ($\Delta\Delta C_T$) method in which the C_T difference (ΔC_T) between target and reference sequences for each individual is compared with the ΔC_T value for control individuals that are known to have two copies of the target sequence. All CNVs were confirmed. To calculate the frequency of an individual event, CNVs were deemed the same if the overlap was greater than or equal to 50% of the union of the two events. Only deletions occurring in less than 1% of the Australian sample were considered rare. For information regarding location, frequency, and genes implicated, for all the large, rare deletions included in the study, see Supplemental Table 1. Healthy controls were genotyped and, as expected, none were found to be carrying a large, rare deletion.

2.3. Clinical assessment

Three clinical factors (positive, negative/disorganized, mood) were computed based on the factor analysis carried out by Fanous et al. (2012) using the Lifetime Dimensions of Psychosis Scale (LDPS) (Levinson et al., 2002). Age at onset was ascertained through medical records and both the Family Interview for Genetic Studies (FIGS) (Maxwell, 1992) and the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al., 1994).

2.4. IQ assessment

The Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999) was used to assess global functioning. It contains four subtests of the full Wechsler Adult Intelligence Scale 3rd Edition (WAIS-III) (Wechsler, 1997) and yields scores for verbal IQ (VIQ), incorporating the vocabulary and similarities subtests, and performance IQ (PIQ) incorporating the block design and matrix reasoning subtests. All four subtests combined yield a full-scale IQ (FSIQ) score.

2.5. IQ analysis

All clinical and cognitive data were analysed using SPSS version 20.0. Analysis of variance (ANOVA) was used to test age differences between the three groups with the chi-square used to test sex differences. All clinical variables were compared using *t*-tests. The IQ tests were compared between healthy controls, general schizophrenia patients and patients with large, rare deletions using ANOVA with Tukey post-hoc analysis.

2.6. Image acquisition

Diffusion tensor imaging (DTI) and magnetic resonance imaging (MRI) were performed using a 3-T Siemens Magnetom TrioTim at the Centre for Advanced Imaging (CAI), University of Queensland. One hundred and ninety-two high-resolution T1-weighted slices were acquired with 0.9 mm^3 resolution, with a repetition time (TR)=1900 ms, echo time (TE)=2.3 ms, inversion time (TI)=900 ms. Acquisition time was 4 min 26 s. DT images were acquired using transverse multislice spin echo, single shot, echo planar imaging (EPI) sequences with a TR=9500 ms, TE=116 ms, and slice thickness of 3 mm with no gap. A 300-mm field of view (FOV) was used with a voxel size of $2.3 \times 2.3 \times 2.5$ mm^3. Diffusion was measured along 64 directions (number of b-values=2, low b-value=0 and high