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Research report

Copy number deletion burden is associated with cognitive, structural, and resting-state network differences in patients with schizophrenia

A.K. Martin[a,*], G. Robinson[b,1], D. Reutens[c,2], B. Mowry[a,d,3]

[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, St Lucia Queensland, 4072, Australia

HIGHLIGHTS

- Negative correlation between total copy number deletion burden and IQ.
- Grey-matter differences associated with total deletion burden.
- White-matter differences associated with total deletion burden.
- Functional connectivity in two key networks is associated with deletion burden.
- Cognitive functioning correlates with functional connectivity.

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ABSTRACT

Total burden of copy number deletions has been implicated in schizophrenia risk and has been associated with reduced cognitive functioning. The current study aims to replicate the cognitive findings and investigate regional grey and white matter volumes. Moreover, it will explore resting-state networks for correlations between functional connectivity and total deletion burden. All imaging differences will be investigated for correlations with cognitive differences. Seventy-eight patients with chronic schizophrenia, who formed a subset of a large genome-wide association study (GWAS), were assessed for intelligence, 34 had structural magnetic resonance imaging, 33 had resting-state functional magnetic resonance imaging, and 32 had diffusion tensor imaging (DTI). Total deletion burden was negatively associated with IQ performance and positively associated with regional volumes in the striatum bilaterally and in the right superior temporal gyrus and white-matter in the corpus callosum. Correlations were identified between deletion burden and both hyper and hypoconnectivity within the default-mode network and hypoconnectivity within the cognitive control network. The functional connectivity correlations with deletion burden were also correlated with the IQ differences identified. Total deletion burden affects regional volumes and resting-state functional connectivity in key brain networks in patients with schizophrenia. Moreover, effects of deletions on cognitive functioning in may be due to inefficiency of key brain networks as identified by dysconnectivity in resting-state networks.

1. Introduction

Schizophrenia is a highly heritable disorder with estimates of heritability as high as 80% [1]. Although recent studies have made considerable ground in explaining these genetic factors, much is left unexplained. The emerging picture is of a complex genetic architecture including both common and rare variants [2]. Of these rare variants, copy number variants (CNVs), especially rare (<1% frequency) deletions have been identified to a greater extent in schizophrenia compared with healthy controls [3]. Recently, several studies have looked to characterize these

* Corresponding author. Tel.:+61 7 3346 3340; fax: +61 7 3346 6006.
E-mail addresses: a.martin11@uq.edu.au (A.K. Martin), g.robinson@psy.uq.edu.au (G. Robinson), cai_pa@uq.edu.au (D. Reutens), b.mowry@uq.edu.au (B. Mowry).
[1] Tel.: +61 7 3365 6401.
[2] Tel.: +61 7 3365 4237.
[3] Tel.: +61 7 3346 6351.

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deletions in schizophrenia. Using a liberal definition of uncommon deletions (<3% frequency), a recent study [4] found cognitive ability to be inversely correlated with deletion burden in patients but not healthy controls, suggesting an interaction between deletions and other schizophrenia associated genetic and environmental risk variants, affecting cognitive functioning. They also found deletion burden to be positively correlated with ventricle size offering evidence that deletions may be impacting structural phenotypes relevant for disease risk. Another study [5] also found a higher rate of deletions that affect genes in patients with schizophrenia compared with healthy controls but found no evidence for an effect of global CNV burden or global deletion burden on total brain volume or grey and white matter volumes. A further study by the same group [6] found no link between total deletion burden and intelligence as measured by the Wechsler Adult Intelligence Scale (WAIS). However, this may be due to the inclusion of all deletions regardless of frequency and could explain the discrepancy between their findings and the findings of Yeo et al. [4].

Although current evidence suggests that total brain volume does not correlate with deletion burden, specific regional effects have not been explored. Regional grey-matter differences in patients with schizophrenia are diffuse and inconsistent between studies [7]. However, a meta-analytical approach of voxel-based morphometry (VBM) studies allowed the comparison of a large number of patients and controls to find regions consistently and robustly associated with disease. A large meta-analysis of 31 studies with 1195 schizophrenia patients and 1262 healthy controls found regional decreases in GM in the insula, anterior cingulate, left parahippocampal gyrus, left middle frontal gyrus, postcentral gyrus, and thalamus. Grey-matter increases were identified in the striatum [8]. Grey-matter volumes are heritable [9], although evidence for their potential as endophenotypes is inconsistent [10,11].

In addition to volume differences, functional and structural connectivity may also provide clues as to how deletions influence the schizophrenia phenotype. Studies of resting-state functional connectivity have primarily identified global hypoconnectivity in patients compared with controls [12] although some studies have provided evidence for distinct patterns of hypo and hyperconnectivity in certain networks, including between striatal and prefrontal regions [13]. Evidence suggests that resting state networks are heritable [14,15] and may be excellent candidates for endophenotypic research [16,17]. Likewise, studies into structural white-matter integrity as measured by diffusion-weighted tensor imaging (DTI) have found differences between patients with schizophrenia and healthy controls [18] and intermediate effects in well family members [19]. Fractional anisotropy (FA) differences are heritable [20] and mutations, especially rare deletions, may influence the connectivity between distinct brain regions rendering the brain less efficient, resulting in risk for psychosis.

In the following study, using a relatively conservative definition of rare deletions (occurring in <1% of our sample), we investigate the relationship between rare deletion burden and IQ, grey-matter as measured by voxel-based morphometry (VBM), white-matter integrity as measured by FA, and resting-state functional connectivity within the cognitive control and default mode networks.

2. Methods and materials

2.1. Participants

Seventy eight patients with schizophrenia were recruited from the Australian subsample of a genome-wide association study [21]. Individuals were comprehensively ascertained by trained clinicians using: (i) the Diagnostic Interview for Genetic Studies (DIGS) [22] (ii) Family Interview for Genetic Studies (FIGS) [23,24]; (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 interview information, especially when the participant's responses lacked clarity; (v) Best Estimate Final Diagnosis (BEFD) [25] was assigned by two experienced research psychiatrists independently reviewing all available information then conferring to assign a consensus diagnosis; one of us (BM) reviewed every Australian case. Diagnostic inter-rater reliability was assessed using standard procedures [26]. Of the 78 patients, 32 were recruited for structural and functional neuroimaging. Of the remaining patients, the loss to follow up was due to being either unwilling or medically incapable of undergoing MRI.

2.2. Copy number variant identification

2.2.1. Original MGS study

Quality control, identification and analytic methods have been described previously [21]. Briefly, 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 the Birdsuite software package [27]. Quality control steps for CNV calls included: 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 [28] pointwise 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 [29] 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 that detect sequence that is 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 to the ΔC_T value for control individuals that are known to have two copies of the target sequence. All CNVs were confirmed. In order 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. To enable confidence in the deletion calls, only those greater than 10,000 bp were included.

2.3. Clinical

Three clinical factors (positive, negative/disorganized, mood) were computed based on the factor analysis carried out by Fanous