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ORIGINAL ARTICLE

Increased rare duplication burden genomewide in patients with treatment-resistant schizophrenia

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Background. A significant number of patients with schizophrenia fail to respond to antipsychotic medication. Although several studies have investigated associated patient characteristics, the emerging findings from genetic studies offer further scope for study.

Method. In 612 schizophrenia patients with detailed clinical information, common genetic variants indexed by polygenic risk scores, and rare variants indexed by deletion and duplication burden genomewide, we explored potential genetic predictors alongside other established risk factors for treatment resistance. Clinical outcomes of treatment resistance were also calculated using lifetime measures of positive, negative/disorganized and mood symptoms as well as number of hospitalizations and suicide attempts.

Results. Logistic regression models identified a significant relationship between treatment resistance and total duplication burden genomewide, years of formal schooling and age at onset. Clinically, treatment-resistant patients were characterized by greater negative/disorganized and positive symptoms and greater number of hospitalizations.

Conclusions. Taken together, these findings suggest genetic information, specifically the genomewide burden of rare copy number variants, may increase our understanding and clinical management of patients with treatment-resistant schizophrenia.

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Key words: Age at onset, clozapine, copy number variants, mutations, predictive models.

Introduction

Anywhere between 20 and 60 % of patients with schizophrenia fail to respond to typical antipsychotic medication (Kane *et al.* 1988; Meltzer & Kostacoglu, 2001; Miller *et al.* 2006). Treatment-resistant schizophrenia is associated with worse outcomes for patients, including higher rates of suicide (Hassan & De Luca, 2015), a more severe, long-lasting symptom profile (Kane *et al.* 1988; McGlashan, 1988; Hassan & De Luca, 2015), and considerable burden to the health system (Revicki *et al.* 1990) due, in part, to increased hospitalizations (McGlashan, 1988). Treatment-resistant schizophrenia can be treated with the atypical antipsychotic clozapine (Chakos *et al.* 2001; Leucht *et al.* 2013); however, due to clozapine's potentially severe adverse drug reactions, it can only be prescribed after failed trials of two other antipsychotic medications (Nielsen *et al.* 2012). Untreated psychosis leads to a poorer prognosis and any information on the likelihood of a patient being treatment resistant has the potential to improve clinical management and outcome. Several variables have been reported as associated with treatment-resistant schizophrenia, perhaps the most robust being poor pre-morbid social functioning and a longer duration of untreated psychosis (Schennach *et al.* 2012). Other factors such as male gender (Lieberman *et al.* 1993; Murray & Van Os, 1998), history of drug or alcohol abuse (Gupta *et al.* 1996), earlier age at onset (Hollis, 2000; Reichert *et al.* 2008; Hassan & De Luca, 2015), and recently lifetime adversity (Hassan & De Luca, 2015), have been associated with treatment-resistant schizophrenia. Moreover, several studies have identified a relationship with family history of psychosis (Murray & Van Os, 1998; Malaspina *et al.* 2000; Crespo-Facorro *et al.* 2013; Hassan & De Luca, 2015), suggesting a genetic component in the development of treatment resistance.

Schizophrenia is a highly heritable disorder with heritability estimates as high as 80% (Cardno & Gottesman, 2000). Large genomewide association studies (GWAS) have begun the search for genetic variants associated with risk with results highlighting the sobering complexity of genetic risk. Although much of the heritability remains to be explained, several advances have been

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made. In the latest GWAS (Schizophrenia Working Group of the Psychiatric Genomics, 2014) including 36 989 cases and 113 075 controls, 108 genome-wide significant loci were identified, highlighting the polygenic nature of risk. Another approach has analysed the variance explained by all variants surpassing an increasingly liberal threshold of association, as there is thought to be several variants that current GWAS do not have power to detect (Lee *et al.* 2012). Using the approach employed by Lee *et al.* (2012), polygenic risk scores (PRS) were calculated from a discovery sample and used to predict into an independent target sample with a threshold of $p < 0.1$. This threshold was identified to have the greatest predictive power when using a meta-analysis of previous GWAS as the target sample and the Molecular Genetics of Schizophrenia (MGS) dataset as the discovery sample (Schizophrenia Working Group of the Psychiatric Genomics, 2014). In addition to common variants, rare structural variants have also been associated with schizophrenia risk (Mowry & Gratten, 2013) as well as clinical and cognitive phenotypic differences in patients with schizophrenia (Martin *et al.* 2014*a*, 2015*b*). Aggregate measures of copy number variant (CNV) burden have been associated with differences in clinical (Yeo *et al.* 2013; Martin *et al.* 2015*a*), cognitive (Yeo *et al.* 2013, 2014; Martin *et al.* 2014*c*), and neuroimaging domains (Martin *et al.* 2014*b*, *c*) and are potentially of interest in genetic studies of treatment resistance.

Several studies have investigated associations between candidate genes and treatment response to typical antipsychotics in first-episode patients with mixed results (Lencz *et al.* 2006; Dolzan *et al.* 2008; Ikeda *et al.* 2008; Rasmussen *et al.* 2011; Zhang & Malhotra, 2011). However, to our knowledge, only one study has investigated the effects of aggregate genetic risk scores. A recent study implicated PRS in treatment resistance in schizophrenia, with earlier onset and poor pre-morbid functioning strengthening the association (Frank *et al.* 2015). In the current study, associations between treatment-resistant schizophrenia and both PRS and total deletion and duplication burden genomewide were analysed. In order to assess whether other predictive variables confound the relationship, a logistic regression, including the genetic variables and relevant developmental and demographic variables, was conducted. Finally, the clinical outcomes associated with treatment resistance were also explored.

Method

Participants

A total of 612 schizophrenia patients who formed the Australian subset of the MGS cohort were included in the analysis. All had genetic information regarding common genetic variants and rare structural variants due to their involvement in large GWAS (Levinson *et al.* 2011; International Schizophrenia Consortium, 2014).

Treatment resistance

The criteria for treatment resistance were formulated in consultation with the literature (Chakos *et al.* 2001; Leucht *et al.* 2013). The binary coding favoured negative ratings; we acknowledge that some treatment-resistant individuals will be rated 'no', but we are confident that all individuals who rate 'yes' are treatment resistant. The criteria were as follows: (i) two or more of delusions, hallucinations, disorganization, and negative symptoms; (ii) moderate or severe current global assessment of functioning; (iii) continuous course of illness; (iv) moderate to severe pattern of disease severity; (v) current antipsychotic medication at the time of assessment.

Genetic variables

PRS

PRS were computed from a discovery sample of 34 351 schizophrenia cases and 110 593 controls (Schizophrenia Working Group of the Psychiatric Genomics, 2014) and calculated for each individual in an independent target sample of 612 cases, with detailed clinical information available. A detailed outline of the method and rationale for PRS is provided in Wray *et al.* (2014). In brief, a GWAS is conducted in a discovery sample. The risk alleles and their effect sizes are then used to generate PRS based on a specified p value threshold of <0.1. The PRS is calculated for each individual in the target sample as the sum of the number of risk alleles weighted by their effect size.

CNV identification

Original MGS study. Quality control, identification and analytic methods have been described previously (Levinson *et al.* 2011). 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 (Korn *et al.* 2008). 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 rather than distributed across several plates, suggesting a plate-specific artifact. DNA samples were also subject to quality-

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control steps. This involved the exclusion of: (i) DNA samples with CNV numbers greater than three standard deviations above the group mean; (ii) samples with more than two chromosomes with outlier calls; (iii) data for outlier chromosomes for subjects with one or more such chromosomes; and (iv) samples with probe intensity variances four standard deviations above the group mean. Plots of 'regions of interest' calls were visually inspected with confirmation by a second calling algorithm, an array-based comparative genomic hybridization (Lai *et al.* 2008). Quantitative polymerase chain reaction (qPCR) confirmed the presence of selected CNVs with evidence for individual association with schizophrenia risk ($n = 13$). PLINK (Purcell *et al.* 2007) pointwise analyses were conducted for all rare CNVs (with <1% frequency in the MGS sample) and those of more than 100 000 base pairs (bp).

Australian MGS schizophrenia subset. Most MGS DNAs were extracted from Epstein–Barr (EB) 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 that detect sequences 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 known to have two copies of the target sequence. 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 and duplications 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.

CNV variables

Following the identification processes detailed above, the following CNV variables were included in the analysis:

Total deletion burden – the total number of base pairs affected by copy number deletions that occur in less than 1% of the sample and that individually affect 10 000 bp or more;

Total duplication burden – the total number of base pairs affected by copy number duplications that occur in less than 1% of the sample and that individually affect 10 000 bp or more.

Clinical variables

Clinical information was attained through structured interviews using the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger *et al.* 1994) and the Family Interview for Genetic Studies (FIGS) (Maxwell, 1992) as well as a thorough search of medical records. A psychiatrist (B.M.) checked and confirmed all diagnoses. Symptom measures were drawn from scores on the Lifetime Diagnosis of Psychosis Scale, organized into three factors (positive, negative/disorganized, and mood) based on a factor analysis from a previous study (Fanous *et al.* 2012).

Family history of psychosis was coded as 'yes' or 'no' based on any presence of psychosis within two degrees of the subject as reported in both DIGS and FIGS with subject and family. Substance or alcohol abuse/dependence was coded according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV; American Psychiatric Association, 1994). Coding was 'yes' or 'no' based on any diagnosis of substance or alcohol abuse/dependence. Abnormal birth or early development was categorized as 'yes' if definite evidence of clinically significant birth complications during the individual's birth, or definite delayed developmental milestones from birth to age 6 years. This information was taken from the diagnostic narrative summary, medical records, DIGS and FIGS. If any doubt, abnormal birth or development was coded as 'no'.

Statistical analysis

Initial differences were analysed using *t* tests or χ^2 tests as appropriate. In the logistic regression models, the genetic variables were modelled separately followed by a model including other predictor variables that may confound the association. Likewise, logistic regression models analysed the relationship between treatment resistance and psychosis-related outcome measures both individually (unadjusted) and with all significant predictor variables (adjusted), in order to account for possible confounding effects.

Ethical standards

All procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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Table 1. *Demographic, genetic and developmental characteristics of treatment-resistant and non-treatment-resistant patients*

			Unadjusted model[a]		Adjusted model[a]	
	Non-treatment resistant ($n = 385$) (62.91%)	Treatment resistant ($n = 227$) (37.09%)	OR (95% CI)	p	OR (95% CI)	p
Genetic						
Polygenic risk score	12.19 (2.70)	12.41 (2.62)	1.03 (0.97–1.10)	0.325	1.05 (0.98–1.12)	0.181
Deletion load (100 kb)	2.68 (4.17)	2.89 (5.07)	1.01 (0.98–1.05)	0.571	1.02 (0.98–1.05)	0.437
Duplication load (100 kb)	2.74 (4.48)	4.13 (7.30)	1.04 (1.01–1.08)	0.007	1.05 (1.02–1.09)	0.002
Other						
Gender: male, *n* (%)	269 (69.9)	170 (74.9)	1.29 (0.89–1.86)	0.183	0.82 (0.55–1.21)	0.312
Family history of schizophrenia, *n* (%)	96 (24.9)	57 (25.1)	1.01 (0.69–1.47)	0.961	0.96 (0.65–1.42)	0.829
Age at onset, years	22.63 (6.75)	21.61 (5.94)	0.98 (0.95–1.00)	0.059	0.97 (0.94–0.99)	0.027
Substance/alcohol abuse, *n* (%)	217 (56.4)	138 (60.8)	1.20 (0.86–1.68)	0.284	1.04 (0.73–1.49)	0.836
Years of schooling	11.22 (2.18)	10.55 (2.21)	0.87 (0.80–0.94)	<0.001	0.86 (0.79–0.93)	<0.001
Abnormal birth/early development, *n* (%)[b]	92 (28.0)	50 (28.4)	1.02 (0.68–1.53)	0.932	1.08 (0.71–1.65)	0.729
DUP, months	10.68 (36.12)	11.16 (34.20)	1.01 (0.95–1.06)	0.870	0.99 (0.94–1.05)	0.834

Data are given as mean (standard deviation) unless otherwise indicated.

OR, Odds ratio; CI, confidence interval; kb, kilobases; DUP, duration of untreated psychosis.

[a] The unadjusted model contains only the variable in question. The adjusted model contains all predictor variables except abnormal birth/early development due to reduced sample size and non-significant unadjusted relationship.

[b] Calculated on a total sample of 504.

Results

In the current sample of 612 schizophrenia patients, 227 (37.09%) were treatment resistant. Although age was slightly higher in the treatment-resistant group (40.75 *v.* 38.91 years), this was non-significant. The two groups were comparable on gender, with 74.95% of the treatment-resistant group male and 69.8% of the non-treatment resistant (see Table 1). PRS was negatively correlated with duplication burden ($r = -0.095$, $p = 0.019$). The relationships between PRS and deletion burden ($r = 0.068$, $p = 0.095$) and between deletion and duplication burden ($r = -0.022$, $p = 0.595$) were both not significant.

The first logistic regression model identified a relationship between total genomewide duplication burden and treatment resistance [odds ratio (OR) 1.04, 95% confidence interval (CI) 1.01–1.08, $p = 0.007$] but not for PRS (OR 1.03, 95% CI 0.97–1.10, $p = 0.325$). In order to assess whether this relationship was mediated by other variables, a logistic regression was carried out including variables with previous evidence for an association with treatment resistance. Table 1 displays both the uncorrected and corrected models including the genetic measures and other possible predictive variables. After the adjustment for all confounders, the association between total genomewide duplication burden and treatment resistance remained significant (OR 1.05, 95% CI 1.02–1.09, $p = 0.002$). Of the other variables, age at onset (OR 0.97, 95% CI 0.94–0.99, $p = 0.027$) and years of schooling (OR 0.86, 95% CI 0.79–0.93, $p < 0.001$) remained significant predictors of treatment resistance status. The complete model had a classification accuracy of 64.3%, a small increase from the initial accuracy of 63%.

In the second logistic regression model (Table 2), association between treatment-resistant schizophrenia and psychosis-related outcome variables were modelled. Treatment-resistant patients had greater number of hospitalizations and more severe and/or long-lasting positive and negative/disorganized symptom profiles. All remained significant in the final model correcting for other outcome variables.

As treatment-resistant patients had an earlier age at onset, the higher number of hospitalizations was potentially confounded by a longer duration of interest. However, number of hospitalizations remained significantly higher with age of onset included in the model.

Discussion

The purpose of the current study was to explore the association between treatment resistance and common

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Table 2. *Psychosis-related variables of treatment-resistant and non-treatment-resistant patients*

			Unadjusted model[a]		Adjusted model[a]	
	Non-treatment resistant ($n = 385$) (63.28%)	Treatment resistant ($n = 227$) (36.72%)	OR (95% CI)	p	OR (95% CI)	p
Positive	25.79 (7.10)	29.03 (7.32)	1.07 (1.04–1.09)	<0.001	1.07 (1.04–1.10)	<0.001
Negative/ disorganized	16.30 (5.60)	18.73 (6.40)	1.07 (1.04–1.10)	<0.001	1.06 (1.03–1.10)	<0.001
Mood	5.72 (5.58)	4.97 (5.62)	0.97 (0.95–1.00)	0.089	0.99 (0.96–1.02)	0.321
Suicide attempt, n (%)[b]	138 (36.0)	99 (43.6)	1.37 (0.98–1.92)	0.064	1.38 (0.98–1.95)	0.063
Hospitalizations[c]	7.42 (11.06)	10.19 (13.22)	1.03 (1.01–1.05)	0.011	1.02 (1.00–1.04)	0.028

Data are given as mean (standard deviation) unless otherwise indicated.

OR, Odds ratio; CI, confidence interval.

[a] The unadjusted model contains only the variable in question. The adjusted model contains all significant predictor variables (age at onset, duplication burden genomewide, and years of formal schooling).

[b] Suicide data missing for two cases.

[c] Hospitalization data missing for five cases.

and rare aggregate genetic variants as indexed by PRS and total genomewide deletion and duplication burden. In contrast to previous findings (Frank *et al.* 2015), we found no association between treatment resistance and PRS. However, we did identify an association for total copy number duplication burden genomewide. Of the other predictive variables, an association was identified for years of schooling and age at onset. In the logistic regression model, duplication burden remained significant even after strict Bonferonni correction for multiple comparisons. Of the other variables, years of schooling also remained significant. The current study supports previous findings (Hollis, 2000; Reichert *et al.* 2008; Hassan & De Luca, 2015) regarding an association between earlier age at onset and treatment resistance. Closer inspection of the data provided no evidence that this was due to a greater number of early-onset schizophrenia cases (<18 years of age) or very early-onset cases (<14 years of age). Therefore, within the normal age at onset range, those whose symptoms develop earlier are more likely to be treatment resistant. In contrast to previous studies we failed to identify a relationship between treatment resistance and gender, duration of untreated psychosis, family history of psychosis, substance or alcohol abuse, or abnormal birth or early development. As for psychosis-related characteristics of treatment resistance, the current study supports previous observations regarding greater severity of symptoms and hospitalizations (McGlashan, 1988; Hassan & De Luca, 2015). However, we failed to replicate a previous association with greater attempted suicide (Hassan & De Luca, 2015), although we observed a trend ($p = 0.06$).

Although the current study failed to provide support for an association between PRS and treatment resistance, the association between total duplication burden genomewide supports a genetic influence on treatment resistance. Although duplication burden is not currently associated with risk for schizophrenia (Buizer-Voskamp *et al.* 2011), it may influence treatment response or be implicated in risk for a certain subset of patients characterized by treatment resistance, through, as yet, unknown mechanisms. Schizophrenia is a heterogeneous disorder almost certainly influenced by a number of contributing biological pathways (Sullivan & Posthuma, 2015). The current results suggest that an increased burden of duplications genomewide influences response to typical antipsychotics and should be considered in future pharmocogenetic studies that aim to improve the clinical decision-making based on genetic factors. It should be noted that the final logistic regression model, with all variables included, only marginally increased the success of predicting treatment resistance, rendering it ineffectual for clinical purposes. However, larger studies and possibly more refined measures of CNV burden and PRS variables may result in greater predictive capability and have a measurable impact in the clinic.

In the current dataset we observed no association for several variables previously implicated in treatment resistance. Gender, family history of psychosis, co-morbid substance or alcohol abuse, duration of untreated psychosis, and abnormal birth or early development all failed to significantly predict treatment resistance. A study with a similar sample size to the current study also identified null findings for gender, substance/alcohol abuse and family history of

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psychosis (Frank *et al.* 2015). However, in contrast to their results, we identified no relationship between treatment resistance and PRS. Although the PRS in the current study were calculated using a threshold of $p < 0.1$ which is more conservative than the $p < 0.5$ threshold used in Frank *et al.* (2015), the current evidence suggests that PRS offer no greater predictive capability when using p thresholds greater than $p < 0.05$ (Schizophrenia Working Group of the Psychiatric Genomics, 2014). Therefore, we are confident that the PRS reported here are comparable in predictive capability to those used in the Frank *et al.* (2015) study. Although comparable in size to Frank *et al.* (2015), both studies would benefit from greater sample size and large collaborative efforts may be required. Larger sample sizes would also allow a more refined analysis of other developmental predictors as well as clinical outcome measures. However, the results from the current study suggest future studies should look at both PRS and CNV burden measures.

In sum, this is the first study to incorporate both PRS and CNV burden measures into predictive models of treatment resistance in schizophrenia. As considerable reduction in the cost to health care systems and improved outcomes for patients are dependent on compliance and response to treatment, understanding the environmental and genetic predictors of treatment response is vital. Evidence from this study suggests that genetic factors may be important, with an emphasis on genomewide duplication burden. Although future research is required to identify the mechanisms of this association, larger and more refined studies of genetic predictors of treatment resistance promise to improve the outcome of a large proportion of patients with schizophrenia.

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Declaration of Interest

None.

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