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Phenotypic features of patients with schizophrenia carrying de novo gene mutations: A pilot study

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ABSTRACT

Genome-wide scans have revealed a significant role for de novo copy number variants (CNVs) and Single Nucleotide variants (SNVs) in the genetic architecture of schizophrenia. The present study attempts to parse schizophrenia based on the presence of such de novo mutations and attempts genotype–phenotype correlation. We examined phenotypic variables across three broad categories: clinical presentation, premorbid function, disease course and functional outcome and compared them in individuals with schizophrenia carrying either a de novo CNV, a de novo SNV, or no de novo mutation. Work skills were worst affected in patients carrying de novo CNVs. More learning disabilities were found in subjects carrying de novo SNVs. Patients with either mutation had older parents at birth and worse functional outcome as measured by SLOF scores. We found no relation between treatment resistance and the presence of de novo mutations. The combined consideration of the functional outcome scores and early deviant behaviours was found to have higher predictive value for underlying genetic vulnerability. Due to the rare nature of the de novo mutations the sample sizes studied here were small. Despite this, valuable phenotypic characteristics were identified in schizophrenia patients carrying de novo mutations and studying larger samples will be of interest.

1. Introduction

Schizophrenia is a leading cause of disability worldwide and is both highly heritable and highly genetically heterogeneous (Rodriguez-Murillo et al., 2012). Advances in genomics have enabled the next generation of studies into the genetics of schizophrenia. Rare gene-disrupting Copy Number Variants (CNVs), Single Nucleotide Variants (SNVs) and small insertions and deletions (indels) have been found to contribute substantially to the disorder (McClellan and King, 2010; Walsh et al., 2008; Xu et al., 2008, 2011, 2012; Stefansson et al., 2008; Gulsuner et al., 2013) and carry high pathogenicity value in their de novo form. A de novo mutation is a genetic alteration that is present for the first time in one family member as a result of a mutation in a germ cell (ovum or sperm) of one of the parents or in the embryo itself. Genes disrupted in schizophrenia and schizoaffective disorder may be revealed by de novo mutations in affected persons from otherwise healthy families (sporadic cases).

We have previously conducted genome-wide scans for de novo CNVs and SNVs in a well-characterized cohort of trios of Afrikaner families in South Africa consisting of individuals affected with schizophrenia and their biological parents. These scans revealed a significant role of de novo CNVs and SNVs in the genetic architecture of schizophrenia (Xu et al., 2008, 2009, 2011, 2012).

In the present study, we aim to determine whether phenotypic differences could be identified among three groups of patients with schizophrenia as stratified by their de novo mutational status. We compare patients who carry a de novo CNV (Group A), to patients who carry a de novo SNV (Group B), to patients carrying no detectable de novo mutations (Group C). We examined phenotypic variables across three broad categories: clinical variables as a way to assess qualitative differences, premorbid variables as indication of a neurodevelopmental course influenced by the presence of a de novo mutation; disease course and functional outcome as a means to determine whether presence of a de novo CNV or SNV is associated with worse outcome.

2. Materials and methods

Subject recruitment: a large number of families with schizophrenia have been recruited from the Afrikaner population over a number of years for a collaborative genetic study (Karayiorgou et al., 2004). The families are of varying structure, and include a large number of trios of families. Each subject underwent a careful,

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in-person diagnostic evaluation using the Diagnostic Interview for Genetic Studies (DIGS) at recruitment (Nurnberger et al., 1994).

A subset of probands from this sample was re-contacted for participation in the current study by the principal clinical investigator of the collaborative study. Follow-up evaluations were performed by two senior registrars (psychiatrists in training) under the supervision of the principal clinical investigator:

- A new diagnostic interview using the DIGS was conducted in order to confirm diagnosis stability since initial recruitment (Nurnberger et al., 1994).
- A Specific Level of Functioning assessment scale (SLOF) was completed by a caregiver (Schneider and Streuning, 1983).
- A checklist on early deviant behaviour in the first 10 years of life was completed (Sobin et al., 2003).
- Other relevant data were collected, including present medication and parents' age at birth. Information was obtained by family members where the patient could not give details.

The senior registrars were blind to the original recruitment DIGS summary reports as well as the genetic status and grouping of the patients. The principal clinical investigator was either present at the follow up interviews or reviewed and discussed the findings of their interviews with the registrars. He was also blind to the genetic status and grouping of the patients.

Ethical approval for this study was obtained from the Ethics Committee of the Faculty of Health Sciences at the University of Pretoria.

A total of 24 patients were identified for participation in this study (7 from Group A, 8 from Group B, and 9 from Group C). Two patients from Group B were lost to follow up. The sample sizes in this study were determined by the number of subjects previously identified to carry de novo CNVs (Group A in this study). In our Xu et al. (2008) study, of 152 subjects that were included in our genome-wide microarray scan, a total of 15 subjects were identified to carry one or more de novo CNV. Of this 15, two were excluded in the present study because they carried at least 2 de novo CNVs; another three were excluded because they carried deletions in chromosome 22q11.2, which occurs recurrently and is being studied separately. Of the remaining 10 subjects carrying de novo CNVs, we were able to recontact 7 probands successfully and secure their participation in the present study (Group A). The remaining groups (B and C) were formed to match Group A in size.

A detailed Table is provided in Appendix A listing the exact chromosomal location of each CNV and SNV, as well as the specific genes disrupted. In addition, age of onset, as well as the number of all de novo CNVs or SNVs per subject is provided.

2.1. The Specific levels of Functioning (SLOF) Scale

The SLOF Scale was the best rated scale by the Validation of Everyday Real-World Outcomes (VALERO) study, (Harvey et al., 2011).

SLOF is a 43-item multidimensional behavioural survey administered in person to the caseworker or caregiver of a schizophrenic patient. The scale assesses the patient's current functioning and behaviour across 6 domains: (1) – physical functioning; (2) – personal care skills; (3) – interpersonal relationships; (4) – social acceptability; (5) – activities of community living; and (6) – work skills. Each of the questions is rated on a 5-point Likert scale and total scores range from 43 to 215. The higher total score, the better the overall functioning of the patient (Schneider and Streuning, 1983).

2.2. Early deviant behaviour checklist

The early childhood behaviour questionnaire probes seven areas of possible deviance including social dysfunction (avoidance of other children, inability to have friends, isolated play), extreme odd behaviours (unprovoked screaming fits, disorganised or irrational behaviour, inappropriate affect), unprovoked aggression, extreme anxiety, chronic sadness, attentional impairment and learning disabilities (Sobin et al., 2003).

2.3. Statistical methodology

Due to the nature of the study, limited patients were available to include in the sample. Since the sample size is small, the assumption of normality necessary for parametric tests could not be verified and is probably violated. Hence statistical data analysis was performed by utilising permutation tests. These tests are not based on any underlying assumptions of the distribution of the data (Edgington and Onghena, 2007). The advantage of permutation tests over the more conventional distribution-free tests is that all the original information in the data are used compared to using only the ranks. Another disadvantage of small sample sizes is that the power of the test to detect significant differences is very low. Because of this and the novel nature of this research, it was decided to not only report results that are significant at the conventional 5% level, but also results that are only moderately significant (p-value < 0.10) (Albright et al., 2005). This approach will assist to gain insight and to direct further research in this field of study.

3. Results

3.1. Stability of the diagnoses

The lifetime diagnoses originally assigned to the subjects were remarkably stable across all 3 groups. The initial study diagnosis was made by a best-estimate process using medical records and collateral information. The average number of years to the follow up assessment since the initial recruitment was 10, 12, and 13 years in Groups A, B, and C, respectively. The diagnoses remained the same in all but 2 cases from Group A, 1 case from Group B, and 1 case from Group C. The stability of the diagnoses were confirmed by re-administering the DIGS and collecting other relevant data.

In Group A, one male patient had a dual diagnosis of Asperger Syndrome and schizophrenia at initial assessment. After follow up assessment the diagnosis of Asperger Syndrome was discarded.

The modification of diagnosis from schizophrenia to schizoaffective disorder in one patient in each of the 3 groups was done because the longitudinal course of the illness was taken into account and a more accurate picture of the mood syndrome was available at the follow up evaluation. The reliability coefficients for schizoaffective disorder are lower than for other diagnoses made in the DIGS (Nurnberger et al., 1994). It remains difficult to assess

Table 1
Specific level of functioning (SLOF) scores.

	Group A ($n=7$)		Group B ($n=6$)		Group C ($n=9$)		
	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.	p-Value
Self-maintenance							
(a) Physical functioning	24.7	0.8	24.3	1.2	24.4	0.7	0.769
(b) Personal care skills	32.7	3.5	32.3	3.8	32.8	2.8	0.968
Sum of (a) and (b)	57.4	3.5	56.7	3.9	57.2	2.8	0.922
Social functioning							
(c) Interpersonal relationships	19.1	6.6	21.3	6.6	22.8	6.3	0.541
(d) Social acceptability	32.0	2.1	30.8	3.8	32.0	4.5	0.826
Sum of (c) and (d)	51.1	6.0	52.2	8.4	54.8	9.4	0.658
Community living skills							
(e) Activities	40.7	12.6	45.3	9.8	48.7	7.4	0.311
(f) Work skills	13.9	4.0	19.0	5.3	20.1	6.8	0.102
Sum of (e) and (f)	54.6	14.0	64.3	13.3	68.8	13.7	0.143
Total SLOF	163.1	18.2	173.2	23.3	180.8	24.2	0.310