

Copy number variants in a sample of patients with psychotic disorders: is standard screening relevant for actual clinical practice?

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Abstract: With the introduction of new genetic techniques such as genome-wide array comparative genomic hybridization, studies on the putative genetic etiology of schizophrenia have focused on the detection of copy number variants (CNVs), ie, microdeletions and/or microduplications, that are estimated to be present in up to 3% of patients with schizophrenia. In this study, out of a sample of 100 patients with psychotic disorders, 80 were investigated by array for the presence of CNVs. The assessment of the severity of psychiatric symptoms was performed using standardized instruments and ICD-10 was applied for diagnostic classification. In three patients, a submicroscopic CNV was demonstrated, one with a loss in 1q21.1 and two with a gain in 1p13.3 and 7q11.2, respectively. The association between these or other CNVs and schizophrenia or schizophrenia-like psychoses and their clinical implications still remain equivocal. While the CNV affected genes may enhance the vulnerability for psychiatric disorders via effects on neuronal architecture, these insights have not resulted in major changes in clinical practice as yet. Therefore, genome-wide array analysis should presently be restricted to those patients in whom psychotic symptoms are paired with other signs, particularly dysmorphisms and intellectual impairment.

Keywords: schizophrenia, psychotic disorders, microarray, copy number variants, 1q21, 7q11.2, 1p13.3

Introduction

It has become obvious that the phenotypical presentation of psychotic disorders such as schizophrenia is extremely heterogeneous and that their symptoms may comprise the entire spectrum of psychopathology with high interindividual variation.^{1,2} Although the diagnostic boundaries of schizophrenia still remain unclear, the worldwide lifetime prevalence of this psychotic disorder is estimated to be 0.5%–1%.³ Previous studies have shown a heritability varying from 40%–70%,⁴ and a family history of schizophrenia has been demonstrated to be the far most important risk factor.^{5,6} Over the past years, substantial evidence has emerged concerning the relevance of gene–environment interactions for the onset and course of schizophrenia.^{7,8}

With the ongoing developments in genetic techniques, particularly array-based comparative genomic hybridization analysis, it has become possible to investigate the human genome in far greater detail than is possible with routine cytogenetic analysis, leading to the discovery of previously undetectable defects.⁹ These so-called copy number variants (CNVs) are deletions or duplications ranging in size from 1 kb to 3 Mb resulting in loss or gain of a DNA segment. De novo microdeletions are generally considered of clinical significance and are frequently associated with

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intellectual disability.¹⁰ Of these patients, a substantial percentage develop symptoms from the affective and/or psychotic cluster after adolescence. Microdeletions and duplications may also be causatively involved in patients with normal intelligence and psychiatric disorders like schizophrenia^{11,12} or autism.^{13,14} In his review, Kirov¹⁵ calculated a collective rate of 3% of patients with schizophrenia in whom rare deletions in 1q42, 22q11, and 1q21 and duplications in 16p are found. In the general population, this percentage is estimated to be 0.1%–1%.¹⁶ The explanatory power of CNVs for the pathophysiology of psychiatric disorders in general and for their phenotypical presentation, however, still needs to be clarified.^{17,18}

In the present study, genome-wide array analysis was performed in a group of patients with carefully diagnosed psychotic disorders in order to detect possible pathogenic CNVs. If present, their phenotypical relevance was explored and discussed.

Methods

Patients and assessments

Over a period of 30 months, 100 patients referred to the Vincent van Gogh Institute for Psychiatry for treatment of psychotic symptomatology were investigated. Patients with previously demonstrated cytogenetic aberrations and/or intellectual disability (IQ < 70) were excluded. According to the medical ethical guidelines, 80 patients gave written informed consent and 20 refused further participation (CCMO registration number: NL20469.097.07). Assessment of symptomatology and collection of data from history was performed using Comprehensive Assessment of Symptoms and History (CASH),¹⁹ Positive and Negative Syndrome Scale (PANSS),²⁰ and Clinical Global Impression Scale (CGI).²¹ Final clinical diagnoses were made in a so-called Longitudinal Evaluation using All Data (LEAD) conference.²² Subsequently, patients were classified according to the International Statistical Classification of Disease and Related Health Problems, 10th Revision (ICD-10) criteria.²³ The main characteristics of the patients including the classification of their psychotic disorders are presented in Table 1. Pictures were taken of all patients for evaluation of dysmorphisms by an experienced clinical geneticist (IF). In case CNVs were found by array, the patient was examined in detail by the clinical geneticist.

Genetic analyses

DNA was isolated from an ethylenediaminetetraacetic acid (EDTA) blood sample and genome wide array analysis was performed with an average resolution of 200 kb using the

Table 1 Main characteristics of the patients (n = 80)

	N	%
Male/female	53/27	66/34
Mean age/range (years)	35.0/18–62	
Mean age at onset of psychosis/range (years)	27.1/9–61	
Mean number of episodes/range	2.8/1–15	
Diagnoses (ICD-10)		
Schizophrenia	50	62.5
Schizoaffective disorder	6	7.5
Acute and transient psychotic disorder	11	13.8
Bipolar disorder (psychotic)	6	7.5
Other psychotic disorders	7	8.8
PANSS scores (range) at inclusion		
Total score	86.1 (46–138)	
Positive subscale	23.1 (11–37)	
Negative subscale	19.9 (7–40)	
Global subscale	43.0 (22–67)	
Mean CGI score (range)		
Severity	4.5 (2–7)	

Abbreviations: PANSS, Positive and Negative Syndrome Scale; CGI, Clinical Global Impression Scale; ICD-10, International Statistical Classification of Disease and Related Health Problems, 10th Revision.

Affymetrix 250 k SNP array platform (Affymetrix, Santa Clara, CA) as described previously.²⁴

Results

As Table 1 shows, 50 patients fulfilled the criteria for schizophrenia (F20) and in 11 patients, a diagnosis of acute and transient psychotic disorder was made (F23). A diagnosis of schizoaffective disorder (F25) was established in six patients whereas in another six, bipolar affective disorder, current episode manic with psychotic symptoms, was present (F31.2). In the remaining seven patients, various diagnoses were made (F29, n = 1; F28, n = 2; F22, n = 3; F21, n = 1). All patients received antipsychotic medication according to the hospital standards.

If screening for dysmorphic features suggested a particular monogenetic disorder, specific genetic tests were performed. However, no abnormalities were disclosed. In 77 patients, microarray analysis did not reveal any abnormalities. In three patients, a submicroscopic chromosome imbalance was detected.

Patient A presented with a subacute onset of psychotic symptoms (total scores on PANSS and CGI-S were 79 and 4, respectively) that remitted within 1 month after treatment with 2.5 mg haloperidol. Her history disclosed no major somatic or psychiatric diseases and there was no family load with neuropsychiatric or genetic disorders. In her, an interstitial loss of 3.5 Mb in 1q21.1 comprising 45 genes was found (Figure 1A). Patient B had a history of a mild and short-lasting psychotic episode 1 year before and did

not use any psychotropics. Apart from atypical psychotic symptoms, there were mild manic symptoms (total scores on PANSS and CGI-S were 78 and 5, respectively). With a treatment regimen comprising risperidone (4 mg daily) and lithium (1600 mg daily), all symptoms gradually resolved within 3 months. Here, a 375 kb interstitial gain in band p13 of chromosome 1 encompassing two genes was detected (Figure 1B). Patient C had neither a history of any psychiatric or somatic disease nor a family load with neuropsychiatric or genetic disorders. Shortly prior to admission he developed paranoid psychotic symptoms (total scores on PANSS and CGI-S were 63 and 2, respectively) which fully remitted after 1 month of treatment with risperidone 4 mg daily. He had a 1.1 Mb interstitial gain in 7q11.21q11.22 containing 11 genes (Figure 1C).

It remained unclear whether these CNVs had occurred de novo, since in none of the patients blood samples of the parents were available for carrier testing. A concise description of psychiatric and somatic phenotypes as well as ICD-10 classification is presented in Table 2.

Discussion

In three out of the 80 patients with psychotic disorders (3.75%), one loss and two gains > 250 kb were detected which is in accordance with the report by Kirov who calculated a collective percentage of 3% CNVs in schizophrenia.¹⁵

Apart from the recent report by Maiti et al,²⁵ who found three de novo CNVs (two gains and one loss) in 7q11.21 in two families with monozygotic twins discordant for schizophrenia in which, however, the breakpoints of these CNVs do not overlap with those in patient C, there is virtually no literature on the clinical significance. We recently detected a larger, completely overlapping gain of 2.4 Mb in 7q11.21 in a prenatal sample and the healthy father of this fetus with congenital diaphragmatic hernia. This makes it less likely that this gain is of clinical relevance.

With respect to 1p13.3, only Ohtsuki et al reported on a possible association with the *netrin G1 (NTNG1)* gene, located at 1p13.3, in Japanese families.²⁶ This does not, however, apply to patient B, nor does it clarify his symptom profile. Its relevance is further challenged by the detection of an exactly similar gain in 1p13.3 (in addition to a pathogenic, de novo duplication in 10q) in an intellectually disabled patient without any psychiatric symptoms who inherited this gain from his healthy father (unpublished data).

Microdeletions in the 1q21 region have been reported in several genome-wide analyses in large populations.^{27,28} A possible relationship between 1q21 and psychotic symptoms was reported by three research groups.^{29–31} Maiti et al reported on two de novo CNVs in families with monozygotic twins discordant for schizophrenia.²⁵ Several genes are mapped on 1q21, such as the *KCNN3*,³² *NOTCH2NL*,²⁵ and *Connexin 40/50*.³³ Associations of these specific genes with schizophrenia in affected patients so far remain equivocal.

The results from this relatively small study using genome-wide array analysis do not differ essentially from the reported large scale studies. It has to be stressed that the commonly applied diagnostic categories, derived from the ICD or Diagnostic and Statistical Manual of Mental Disorders systems, show no relationship to a specific genetic etiology. Moreover, a single gene defect never codes for a categorical psychiatric disorder, but may lead to a biological dysfunction that has a certain probability to be associated with the development of an array of psychiatric symptoms.^{14,17,18} This can best be illustrated by the discovery of the *DISC1* gene in one family that was originally thought to be causally related to schizophrenia,³⁴ but later appeared to be involved in a biochemical cascade with consequences for neuronal functions predisposing for psychiatric symptoms across diagnostic boundaries.^{35,36}

Although the CNVs detected in three out of 80 patients are not likely to be primarily involved in the evolution of their psychotic disorders, systematic genetic analysis in patients

Table 2 Phenotype of the three patients with a potentially pathogenic CNV

Patient	Psychiatric phenotype	Somatic phenotype	ICD-10 diagnosis	CNV
A (61-year-old female)	Panxiety, religious delusions, delusions of influence, catastrophic thoughts, olfactory and auditory hallucinations	Dysplastic ear helices, length and head circumference <2.5 SD	Acute polymorphic psychotic disorder without symptoms of schizophrenia	3.5 Mb loss in 1q21.1
B (36-year-old male)	Pressure of speech, misrecognition of persons, thought incoherence, transient visual misinterpretations	No dysmorphism	Schizoaffective disorder, manic type	375 kb gain in 1p13.3
C (22-year-old male)	Delusions of persecution and grandiose identity, nonaffective verbal hallucinations about himself	No dysmorphism	Acute schizophrenia-like psychotic disorder	1.1 Mb gain in 7q11.21q11.22

Abbreviations: CNV, copy number variant; ICD-10, International Statistical Classification of Disease and Related Health Problems, 10th Revision; SD, standard deviation.

may reveal novel recurrent microdeletion syndromes that, however, are nearly always accompanied with developmental delay and dysmorphisms, and do not seem to correlate with psychiatric symptoms.^{37,38} The counterpart microduplications in these regions often cause a much milder phenotype.

In conclusion, the results of this and other studies on the presence of CNVs in patients with psychotic disorders have not yet led to a further specification of their pathophysiology nor to breakthroughs in clinical strategies. Therefore, while genetic analysis should always be considered as part of the diagnostic equipment in neuropsychiatry, the application of genome-wide array analysis in patients with psychotic disorders is mandatory only in the presence of other clinical signs such as facial dysmorphisms or developmental delay.

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Disclosure

The authors report no conflicts of interest in this work.

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