Journal of Psychiatric Research 44 (2010) 971–978

Contents lists available at ScienceDirect

Journal of Psychiatric Research

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

# Comprehensive copy number variant (CNV) analysis of neuronal pathways genes in psychiatric disorders identifies rare variants within patients

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ARTICLE INFO

*Article history:*
Received 22 January 2010
Received in revised form
12 March 2010
Accepted 16 March 2010

*Keywords:*
CNVs (copy number variants)
Psychiatric disorders
MLPA
Rare variants
CNS (central nervous system)
candidate genes

ABSTRACT

*Background:* Copy number variations (CNV) have become an important source of human genome variability noteworthy to consider when studying genetic susceptibility to complex diseases. As recent studies have found evidences for the potential involvement of CNVs in psychiatric disorders, we have studied the dosage effect of structural genome variants as a possible susceptibility factor for different psychiatric disorders in a candidate gene approach.
*Methods:* After selection of 68 psychiatric disorders' candidate genes overlapping with CNVs, MLPA assays were designed to determine changes in copy number of these genes. The studied sample consisted of 724 patients with psychiatric disorders (accounting for anxiety disorders, mood disorders, eating disorders and schizophrenia) and 341 control individuals.
*Results:* CNVs were detected in 30 out of the 68 genes screened, indicating that a considerable proportion of neuronal pathways genes contain CNVs. When testing the overall burden of rare structural genomic variants in the different psychiatric disorders compared to control individuals, there was no statistically significant difference in the total amount of gains and losses. However, 14 out of the 30 changes were only found in psychiatric disorder patients but not in control individuals. These genes include *GRM7*, previously associated to major depression disorder and bipolar disorder, *SLC6A13*, in anxiety disorders, and *S100B*, *SSTR5* and *COMT* in schizophrenia.
*Conclusions:* Although we have not been able to found a clear association between the studied CNVs and psychiatric disorders, the rare variants found only within the patients could account for a step further towards understanding the pathophysiology of psychiatric disorders.


## 1. Introduction

Psychiatric disorders embrace a wide range of complex illnesses, which are caused by multiple genes interacting with each other and with environmental factors to create a gradient of genetic liability to disease (Kiberstis and Roberts, 2002; Weeks and Lathrop, 1995). The genetic contribution to these pathologies has been broadly

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doi:10.1016/j.jpsychires.2010.03.007

demonstrated by means of adoption, twin and familial studies, estimating heritability rates to range from 33% in major depression to around 80% in schizophrenia (Kendler, 2001). However, and despite a significant genetic component, the molecular basis of psychiatric disorders remains largely unknown. Throughout the years, several molecular genetic strategies have been used with the aim of untangling the genetics underlying these pathologies, especially through linkage and association studies, and using mainly microsatellites and single nucleotide polymorphisms (SNPs) as genetic markers. A few years ago, the discovery of copy number variations (CNVs) in the human genome opened a new field to explore the genetic variation as a source of susceptibility and CNVs began to be used as genetic markers or functional candidates in gene-mapping studies of complex traits (Estivill and Armengol, 2007).

A CNV is defined as a DNA segment that is one kilobase (kb) or larger and present at a variable copy number in comparison with a reference genome (Feuk et al., 2006). A bulk of studies have reported the widespread presence of this common structural genomic variation not only among healthy human individuals (Conrad et al., 2006; Hinds et al., 2006; Iafrate et al., 2004; McCarroll et al., 2006; Repping et al., 2006; Sharp et al., 2006; Tuzun et al., 2005), but also in other mammals (Perry et al., 2006). The study of healthy control samples through comparative genomic hybridization (CGH), or SNPs genotyping and ultra-sequencing approaches (Eichler et al., 2007; Kidd et al., 2008; Korbel et al., 2007; Redon et al., 2006) has yielded a catalogue of almost 30,000 CNVs and 8410 CNV loci in human populations annotated in the database of genomic variants (http://projects.tcag.ca/variation/).

In a further step, variations in genomic copy numbers have been also linked to complex disease traits such as an increased susceptibility to HIV-1 (human immunodeficiency virus-1) infection, Crohn's disease, and development of systemic autoimmunity or susceptibility to systemic lupus erythematosus, among other disorders (Aitman et al., 2006; Fanciulli et al., 2007; Fellermann et al., 2006; Gonzalez et al., 2005; Yang et al., 2007). The mechanisms by which interindividual changes in CNVs can cause disease include an altered gene dosage (deletion or duplication) affecting the gene expression levels (Buckland, 2003; McCarroll et al., 2006; Nguyen et al., 2006; Repping et al., 2006) or effects on gene structure or regulation (Stranger et al., 2007).

Since CNVs are potential important contributors to disease, the study of variation in CNVs as a possible factor influencing susceptibility to psychiatric illnesses is also an emerging field in which promising results have been obtained in recent years. First, Wilson et al. (2006) reported copy number aberrations present at four loci (three of them contained genes encoding for proteins involved in glutamate signaling) in schizophrenic and bipolar cases but not in control individuals. However, a subsequent study could not replicate these results (Sutrala et al., 2007). Then, Moon et al. (2006) identified 35 aberrant chromosomal regions, accounting for gains and losses of CNVs when analyzing schizophrenic patients, many of which were consistent with previous studies. Later, Lachman et al. (2007) studied the *GSK3β* (glycogen synthase kinase) gene, partially contained in a CNV, in bipolar disorder and unaffected individuals. They found a statistically significant increased number of gains of the *GSK3β* gene in bipolar disorder patients. More recently, different studies have shown a higher overall burden of rare structural genomic variants in different psychiatric disorders when compared to a large number of control individuals, suggesting that some of these disorders may result from the effects of multiple rare structural variants. In this line, Sebat et al. (2007) found spontaneous CNVs to be more frequent in patients with autism spectrum disorder than in unaffected individuals. In the case of schizophrenia, different studies have reported *de novo* structural variants or rare CNVs to be specific or more frequent among patients (ISC, 2008; Kirov et al., 2009; Stefansson et al., 2008; Walsh et al., 2008; Xu et al., 2008). Finally, Zhang et al. (2009,b) found an increased presence of singleton deletions in bipolar disorder cases.

Based on the above evidences of the potential involvement of CNVs in psychiatric disorders, we have studied the dose effect of structural genome variants as a possible susceptibility factor for different psychiatric disorders in a candidate gene approach. With that purpose, we selected CNVs entirely containing or overlapping with genes involved in biological processes of the central nervous system (CNS). We have tested for the occurrence of 68 known CNVs using multiplex ligation-dependent probe amplification (MLPA) assays and performed case–control association analyses in four different psychiatric disorders: schizophrenia, mood disorders, eating disorders and anxiety disorders.

## 2. Materials and methods

### 2.1. Subjects and clinical assessment

The clinical sample used in this study was obtained through a collaborative initiative of the Psychiatric Genetics Network (http//www.rgpg.net), supported by the Carlos III Research Institute in Spain. All participants in this Network collected DNA samples and clinical data corresponding to different psychiatric diagnoses. The clinical sample for the study consisted of 724 patients with different psychiatric disorders (175 anxiety disorder, 180 mood disorder, 178 eating disorder and 191 schizophrenic patients) who fulfilled the corresponding DSM-IV criteria (APA, 2000) for each diagnosis. All individuals were of Spanish descent and were recruited from specialized centers from Catalonia: Bellvitge University Hospital, Corporació Sanitària Parc Taulí, and IMIM-Hospital del Mar, Barcelona. All patients were assessed using the Spanish version of the Structured Clinical Interview for DSM-IV AxisI-Disorders (First et al., 1997). According to DSM-IV codes, the samples were grouped upon four different diagnostic categories: anxiety disorders, eating disorders, mood disorders and schizophrenia. For simplicity, we considered only axis I diagnosis. In case of comorbidity of axis I, we considered the main diagnoses.

The control sample consisted of 341 healthy volunteers recruited from blood donors in the Blood and Tissue Bank from the Catalan Health Service and were matched to cases for ethnicity. Description of both clinical and control sample used in this study is shown in Table 1. Finally, three samples from the Corporació Sanitària Parc Taulí were used as positive controls in MLPA experiments, since they contained known chromosomal rearrangements: one duplication at 22q11.2, one deletion at 22q11.2 and one deletion at 15q11-q13. The study complied with the guidelines of the Medical Ethical Committee from each of the participating institutions. Informed consent was obtained from all participants and anonymity of data was guaranteed.

**Table 1**
Subjects of the study of copy number variants in psychiatric disorders. Demographic and clinical characteristics of the psychiatric patients and control individuals analyzed.

| Diagnosis | N | Sex | | Age (mean ± SD) |
|---|---|---|---|---|
| | | Male (%) | Female (%) | |
| Anxiety Disorders | 175 | 54 (30.9) | 121 (69.1) | 33.61 ± 10.20 |
| Mood Disorders | 180 | 61(33.9) | 119 (66.1) | 59.44 ± 15.09 |
| Eating Disorders | 178 | 5 (2.8) | 168 (94.4) | 25.34 ± 6.36 |
| Schizophrenia | 191 | 154 (80.6) | 37 (19.4) | 33.22 ± 8.43 |
| Controls | 341 | 201 (58.9) | 140 (41.1) | 39.76 ± 11.92 |