Journal of Affective Disorders 186 (2015) 284–292

Contents lists available at ScienceDirect

Journal of Affective Disorders

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

Research report

Association between genetic variants related to glutamatergic, dopaminergic and neurodevelopment pathways and white matter microstructure in child and adolescent patients with obsessive–compulsive disorder

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ARTICLE INFO

Article history:
Received 20 March 2015
Received in revised form
20 July 2015
Accepted 29 July 2015
Available online 30 July 2015

Keywords:
Gene
Polymorphism
Neuroimaging
DTI
OCD
Child

ABSTRACT

Background: Alterations in white matter (WM) integrity observed in patients with obsessive–compulsive disorder (OCD) may be at least partly determined genetically. Neuroimaging measures of WM microstructure could serve as promising intermediate phenotypes for genetic analysis of the disorder. The objective of the present study was to explore the association between variability in genes related to the pathophysiology of OCD and altered WM microstructure previously identified in child and adolescent patients with the disease.

Methods: Fractional anisotropy (FA) and mean diffusivity (MD) measured by diffusion tensor imaging (DTI) and 262 single nucleotide polymorphisms (SNPs) in 35 candidate genes were assessed concomitantly in 54 child and adolescent OCD patients.

Results: Six polymorphisms located in the glutamate transporter gene (*SLC1A1* rs3087879), dopamine transporter gene (*SLC6A3* rs4975646), dopamine receptor D3 (*DRD3* rs3773679), nerve growth factor receptor gene (*NGFR* rs734194 and rs2072446), and cadherin 9 gene (*CDH9* rs6885387) showed significant *p*-values after Bonferroni correction ($p \leq 0.00019$). More specifically, the vast majority of these associations were detected with MD in the right and left anterior and posterior cerebellar lobes.

Limitations: Patients were under pharmacological treatment at the time of the DTI examination. Sample size is limited.

Conclusions: The results provide the first evidence of the involvement of genetic variants related to glutamatergic, dopaminergic, and neurodevelopmental pathways in determining the WM microstructure of child and adolescent patients with OCD, which could be related to the neurobiology of the disorder.

1. Introduction

Obsessive–compulsive disorder (OCD) is a common neuropsychiatric disease that affects between 1% and 3% of the population (Ruscio et al., 2010). It is characterized by recurrent, persistent, and intrusive thoughts or images that cause distress or anxiety (i.e., obsessions), and repetitive behaviors aimed at reducing this feeling of anxiety (i.e., compulsions). This symptomatology is distressing, time-consuming and significantly impairing (American Psychiatric Association, 1994).

The precise etiology of OCD is unknown, but there is evidence of a clear genetic susceptibility, particularly in children and adolescents. Twin and family studies have shown a high degree of heritability, around 50%, of this psychiatric disorder (Mataix-Cols et al., 2013; Tambs et al., 2009). More than 80 genetic association studies of OCD covering candidate genes that could be involved in the pathophysiology and pharmacology of OCD, have been

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http://dx.doi.org/10.1016/j.jad.2015.07.035
0165-0327/

published over the last decade. Although the results are controversial, several genetic associations have been reported with multiple genes involved in different pathways including the serotonergic (e.g. *SCL6A4* or *TPH2*), dopaminergic (e.g. *SLC6A3* or *DRD4*) and glutamatergic pathways (e.g. *SLC1A1* or *GRIN2B*), among others (Nicolini et al., 2009; Walitza et al., 2010).

Neuroimaging studies have shown several alterations in brain regions of OCD patients, including altered functional connectivity among gray matter nodes of the cortico–striato–thalamic circuits (CSTC) (Harrison et al., 2009; Jung et al., 2013), the middle frontal gyrus and anterior cingulate cortex (ACC) (Jang et al., 2010) and temporal regions (Zhang et al., 2011). Diffusion tensor imaging (DTI) is a structural magnetic resonance imaging (MRI) technique that can be used to examine white matter (WM) microstructure in humans. The two main parameters derived from DTI, fractional anisotropy (FA) and mean diffusivity (MD), are quite sensitive to a number of tissue properties, such as axonal ordering, axonal density, or degree of myelination, and most probably reflect alterations in some of these aspects of connectivity. In recent years, a growing number of studies using this technique have been published. Results obtained in OCD patients have shown that apart from alterations in the WM tracts within CSTC, there are more widespread WM abnormalities involving several brain regions such as the corpus callosum, cingulum, internal capsule, parietal, temporal and occipital areas (Koch et al., 2014; Piras et al., 2013). A previous study by our group performed in child and adolescent OCD patients showed altered DTI measures in several clusters involving different brain regions including the anterior region of the corpus callosum, the anterior cingulate gyrus, the medial, right superior and left inferior frontal gyrus, the cerebellum involving the anterior and posterior lobes and the pons, the left lentiform nucleus and the lingual gyrus of the occipital lobe (Lázaro et al., 2014).

We hypothesized that alterations in WM integrity observed in OCD may be at least partly determined genetically. In this regard, neuroimaging measures of WM microstructure could serve as promising intermediate phenotypes for genetic analysis of the disorder. OCD studies in which genetic and neuroanatomical variables are evaluated concurrently are few in number and, to date, DTI has not been performed in any of them (Grünblatt et al., 2014). The objective of the present study was to explore the association between variability in genes related to the pathophysiology of OCD and altered WM microstructure previously identified in child and adolescent patients with the disease. To this end, FA and MD values measured by DTI of clusters in which significant differences were previously found between child and adolescent OCD patients and age matched healthy controls (Lázaro et al., 2014) and 262 single nucleotide polymorphisms (SNPs) in 35 candidate genes were assessed concomitantly in 54 of these OCD patients. These genes and SNPs were previously selected for an early-onset OCD transmission disequilibrium study (Mas et al., 2014).

2. Materials and methods

2.1. Subjects

Eighty-seven patients meeting DSM-IV (American Psychiatric Association, 1994) diagnostic criteria for OCD were recruited from the Department of Child and Adolescent Psychiatry and Psychology at the Hospital Clinic of Barcelona. The age of onset was defined as the age at which patients first displayed significant distress or impairment associated with obsessive–compulsive symptoms. Non-Caucasian patients were excluded.

Genetic and DTI data were obtained from 75 to 63 patients, respectively. Finally, 54 patients, 30 males and 24 females, from whom both genetic and DTI data were available, were included in the analysis (Table 1). Patients were aged from 11 to 18 years (mean ± SD: 15.7 ± 2.1). The age of OCD onset was around 13 years (mean ± SD: 13.3 ± 2.6). OCD symptoms were assessed by the Children's Yale-Brown Obsessive–Compulsive Scale (CY-BOCS) (Scahill et al., 1997). The responses of OCD patients on the CY-BOCS were categorized dichotomously (present/absent). In addition, the principal or most prominent obsession and compulsion was registered. In accordance with Leckman et al. factor analyses (Leckman et al., 1997), we computed the CY-BOCS data for four separate obsessive–compulsive symptom dimensions (aggressive, sexual, and religious obsessions and checking compulsions ($n=34$); symmetry, ordering, counting, and arranging obsessions and compulsions ($n=11$); contamination obsessions and cleaning compulsions ($n=9$); and hoarding) for each subject. A total severity score was also obtained, ranging from 0 to 40, with higher scores indicating greater severity (mean ± SD: 18.1 ± 8.7). An additional Axis I diagnosis was made in 38 OCD patients, with generalized anxiety disorder being the most frequent comorbidity. The comorbid diagnoses were not in an acute phase of disease. With regard to pharmacological therapy, most of OCD patients (87%) were under treatment with selective serotonin reuptake inhibitors (SSRIs).

All procedures were approved by the hospital's Ethics Committee. Written informed consent was obtained from all parents and verbal informed consent was given by all patients following an explanation of the procedures involved.

2.2. Sample preparation

Blood samples from participants were collected in EDTA (K2EDTA BD Vacutainer EDTA tubes; Becton Dickinson, Franklin Lakes, New Jersey) and genomic DNA was extracted with the MagNA Pure LC DNA isolation Kit III and an LC MagNA Pure system

Table 1
Sociodemographic and clinical data of OCD patients included in the study.

	OCD patients
N	54
Male gender, *N* (%)	30 (55.5)
Age (Mean ± SD)	15.7 ± 2.1
Age of onset (Mean ± SD)	13.3 ± 2.6
CY-BOCS (Mean ± SD)	18.1 ± 8.7
Dimension, *N* (%)	
Washing/cleaning	9 (16.7)
Harm/checking	34 (62.9)
Symetry/ordering	11 (20.4)
Comorbid condition[a], *N* (%)	
Generalized anxiety disorder	13 (24.1)
ADHD	6 (11.1)
Anorexia nervosa	5 (9.2)
Tourette disorder	4 (7.4)
Oppositional defiant disorder	3 (5.5)
Hypomania	2 (3.7)
Major depression disorder	2 (3.7)
Panic disorder	1 (1.8)
Bulimia nervosa	1 (1.8)
Social phobia	1 (1.8)

ADHD, Attention Deficit Hyperactivity Disorder.
CY-BOCS, Children's Yale-Brown Obsessive Compulsive Scale.
SD, Standard deviation.
[a] Current or lifetime.