# Brain correlates of obsessive-compulsive symptoms in healthy children

Maria Suñol Rodrigo

# Brain correlates of obsessive-compulsive symptoms in healthy children

Doctoral Thesis by:

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To obtain the degree of Doctor from the University of Barcelona in accordance with the requirements of the international PhD diploma

Supervisors:

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“You cannot get through a single day without
having an impact on the world around you.
What you do makes a difference, and you have to decide
what kind of difference you want to make.”

**Jane Goodall**

L'Hospitalet de Llobregat, 11th November 2020

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CERTIFY that they have guided and supervised the doctoral thesis entitled "Brain correlates of obsessive-compulsive symptoms in healthy children", which is presented in order to obtain the title of doctor by the candidate Maria Suñol Rodrigo. They thereby assert that this thesis fulfills all the required criteria to be defended.

# Table of Content

# Foreword

This thesis has been developed within the framework of the Doctoral Program in Medicine and Translational Research at the University of Barcelona. All the research results included have been developed in the Laboratory of Neuroimaging and Mental Health within the Department of Psychiatry at Bellvitge University Hospital. This unit is linked to the Faculty of Medicine and Health Sciences of the University of Barcelona and is part of the research group in Psychiatry and Mental Health of the Bellvitge Biomedical Research Institute (IDIBELL, Catalan acronym). This group is a consolidated research group of the Government of Catalonia (2017 SGR 1247) and the Biomedical Research Networking Centre in Mental Health (CIBERSAM, Spanish acronym) of the Carlos III Health Institute. Part of the work was conducted in collaboration with the Department of Radiology of the Gordon Center for Medical Imaging at Harvard Medical School-Massachusetts General Hospital, Boston, USA, under the supervision of Prof. Jorge Sepulcre.

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The present thesis includes two published peer-reviewed journal articles and one study in preparation. It has been written in accordance with the procedures indicated by the University of Barcelona and it is presented in order to obtain the International Doctorate award, which is granted by this institution. The supervisors of this thesis are Dr. Carles Soriano Mas and Dr. Josep Manuel Menchón Magriñá.

With regards to the structure of the thesis, after a general introduction on obsessive-compulsive symptoms and a methodology section particularly focused on the different neuroimaging and neuroimaging-gene expression interaction analyses techniques used, results of these three studies are reported in the following order:

1. **Suñol M**, Contreras-Rodríguez O, Macià D, Martínez-Vilavella G, Martínez-Zalacaín I, Subirà M, Pujol J, Sunyer J, Soriano-Mas C (2018). Brain Structural Correlates of Subclinical Obsessive-Compulsive Symptoms in Healthy Children. J Am Acad Child Adolesc Psychiatry, 57(1):41-47. doi: 10.1016/j.jaac.2017.10.016.
2. **Suñol M**, Saiz-Masvidal C, Contreras-Rodríguez O, Macià D, Martínez-Vilavella G, Martínez-Zalacaín I, Menchón JM, Pujol J, Sunyer J, Soriano-Mas C (2020). Brain Functional Connectivity Correlates of Subclinical Obsessive-Compulsive Symptoms in Healthy Children. J Am Acad Child Adolesc Psychiatry. S0890-8567(20)31836-0. doi: 10.1016/j.jaac.2020.08.435. Online ahead of print.
3. **Suñol M**, Alemany S, Bustamante M, Diez I, Contreras-Rodríguez O, Laudo B, Macià D, Martínez-Vilavella G, Martínez-Zalacaín I, Menchón JM, Pujol J, Sunyer J, Sepulcre J, Soriano-Mas C. Neurogenetics of attractor dynamic patterns associated with obsessive-compulsive symptoms in healthy children. (In preparation).

In the final part of the thesis, a general discussion with a summary and an interpretation of the findings is developed. An abstract in Catalan is included with a similar structure to that disclosed and a list of references is attached. Finally, it should be mentioned that the present thesis' results have been presented in several international congresses as poster and abstract publications: 71$^{st}$ Annual Scientific Convention & Program "Illuminating the Scientific Process: Innovation, Replication, and Paradigm Shifts in Biological Psychiatry", May 2016, Atlanta, Georgia, USA; European College of Neuropsychopharmacology (ECNP) Workshop on Neuropsychopharmacology for Junior Scientists in Europe, May 2017, Nice, France; 30$^{th}$ European College of Neuropsychopharmacology (ECNP) Congress, September 2017, Paris, France; 31$^{st}$ European College of Neuropsychopharmacology (ECNP) Congress, October 2018, Barcelona, Spain; 14$^{th}$ Meeting of the International College of Obsessive-Compulsive Spectrum Disorders (ICOCS), October 2018, Barcelona, Spain; 25$^{th}$ Meeting of the Organization for the Human Brain Mapping (OHBM), June 2019, Rome, Italy; 33$^{rd}$ European College of Neuropsychopharmacology (ECNP) Virtual Congress, September 2020.

The research activity developed over these years has led to additional publications not included in the thesis. These articles are the result of collaborative work with other projects during the time of the thesis:

1. **Suñol M**, Martínez-Zalacaín I, Picó-Pérez M, López-Solà C, Real E, Fullana MÀ, Pujol J, Cardoner N, Menchón JM, Alonso P, Soriano-Mas C (2020). Differential patterns of brain activation between hoarding disorder and obsessive-compulsive disorder during executive performance. Psychol Med, 50(4):666-673. doi: 10.1017/S0033291719000515.
2. Steward T, Picó-Pérez M, Mestre-Bach G, Martínez-Zalacaín I, **Suñol M**, Jiménez-Murcia S, Fernández-Formoso JA, Vilarrasa N, García-Ruiz-de-Gordejuela A, Veciana de las Heras M, Custal N, Virgili N, Lopez-Urdiales R, Menchón JM, Granero R, Soriano-Mas C, Fernandez-Aranda F (2019). A multimodal MRI study of the neural mechanisms of emotion regulation impairment in women with obesity. Transl Psychiatry, 9(1):194. doi: 10.1038/s41398-019-0533-3.
3. Bueichekú E, Aznárez-Sanado M, Diez I, d'Oleire-Uquillas F, Ortiz-Terán L, Qureshi AY, **Suñol M**, Basaia S, Ortiz-Terán E, Pastor MA, Sepulcre J (2020). Central neurogenetic signatures of the visuomotor integration system. Proc Natl Acad Sci U S A, 117(12):6836-6843. doi: 10.1073/pnas.1912429117.

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# List of Abbreviations

**5-HT2A**: Serotonin 2A Receptor

**ACC**: Anterior Cingulate Cortex

**AD**: Axial Diffusivity

**ADHD**: Attention Deficit Hyperactivity Disorder

**ADHD-RS-IV**: Attention Deficit Hyperactivity Disorder Rating Scale IV

**AHBA**: Allen Human Brain Atlas

**AMPA**: α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid Receptor

**ASB13**: Ankyrin Repeat and SOCS Box Containing 13 Gene

**ATP1B1**: ATPase Na+/K+ Transporting Subunit Beta 1 Gene

**BDNF**: Brain-Derived Neurotrophic Factor Gene

**BOLD**: Blood-Oxygen-Level-Dependent

**CAPRIN2**: Caprin Family Member 2 Gene

**CDH20**: Cadherin 20 Gene

**CPE**: Carboxypeptidase E Gene

**CSF**: Cerebrospinal Fluid

**CSTC**: Cortico-Striato-Thalamo-Cortical

**CTTNBP2**: Cortactin-Binding Protein 2 Gene

**CY-BOCS**: Children's Yale-Brown Obsessive-Compulsive Scale

**D1/2/3**: Dopamine Receptor 1/2/3

**DAT**: Dopamine Transporter

**DAT1/SLC6A3**: Dopamine Transporter Gene

**DLGAP1**: DLG-Associated Protein 1 Gene

**dlPFC**: Dorsolateral Prefrontal Cortex

**DMN**: Default Mode Network

**dmPFC**: Dorsomedial Prefrontal Cortex

**DRD3/4**: Dopamine Receptor D3/4 Gene

**DSM-V**: Diagnostic and Statistical Manual of Mental Disorders - Fifth Edition

**DTI**: Diffusion Tensor Imaging

**DY-BOCS**: Dimensional Yale-Brown Obsessive-Compulsive Scale

**EEG**: Electroencephalography

**EPI**: Echo Planar Imaging

**eQTLs**: Expression Quantitative Trait Loci

**ESR1**: Estrogen Receptor 1 Gene

**FA**: Fractional Anisotropy

**FAIM2**: Fas Apoptotic Inhibitory Molecule 2 Gene

**FDR**: False Discovery Rate

**fMRI**: Functional Magnetic Resonance Imaging

**FPN**: Frontoparietal Network

**FWE**: Family-Wise Error

**GABA**: ɣ-Aminobutyric Acid

**GM**: Grey Matter

**GNAQ**: G Protein Subunit Alpha Q Gene

**GO**: Gene Ontology

**GPCRs**: Gαq-protein-coupled receptors

**GRIA2**: Glutamate Ionotropic Receptor AMPA Type Subunit 2 Gene

**GRIK2**: Glutamate Ionotropic Receptor Kainate Type Subunit 2 Gene

**GRIN2A/B**: Glutamate Ionotropic Receptor NMDA Type Subunit 2A/B Gene

**GRM7**: Metabotropic Glutamate Receptor 7 Gene

**GTEx**: Genotype-Tissue Expression

**GWAS**: Genome-Wide Association Studies

**HTR2A**: 5-Hydroxytryptamine Receptor 2A Gene

**HTR3E**: 5-Hydroxytryptamine Receptor 3E Gene

**HTTLPR/SLC6A4**: Serotonin-Transporter-Linked Polymorphic Region

**ICA**: Independent Component Analysis

**ICC**: Intrinsic Connectivity Contrast

**IFG**: Inferior Frontal Gyrus

**IFOF**: Inferior Fronto-Occipital Fasciculus

**IOCDF-GC**: International OCD Foundation Genetics Collaborative

**IOP8**: Importin 8 Gene

**LD**: Linkage Disequilibrium

**LF-rTMS**: Low-Rrequency repetitive Transcranial Magnetic Stimulation

**LMX1A**: LIM Homeobox Transcription Factor 1 Alpha Gene

**LN**: Limbic Network

**MAF**: Minor Allele Frequency

**MD**: Mean Diffusivity

**MFG**: Middle Frontal Gyrus

**mGluR**: Metabotropic Glutamate Receptor

**MNI**: Montreal Neurological Institute

**MRI**: Magnetic Resonance Imaging

**MRS**: Magnetic Resonance Spectroscopy

**NHE1**: Na+/H+ Exchanger 1

**NKA**: Na+/K+ ATPase

**NMDA**: N-Methyl-D-Aspartate Receptor

**NRXN1**: Neurexin 1 Gene

**NTRK**: Neurotrophic Receptor Tyrosine Kinase 3 Gene

**OBIC**: OCD Brain Imaging Consortium

**OBQ**: Obsessive-Beliefs Questionnaire

**OCD**: Obsessive-Compulsive Disorder

**OCGAS**: OCD Collaborative Genetics Association Study

**OCI**: Obsessive-Compulsive Inventory

**OCI-CV**: Obsessive-Compulsive Inventory Child Version

**OCI-R**: Obsessive-Compulsive Inventory Revised

**OFC**: Orbitofrontal Cortex

**Padua-IR**: Padua Inventory Revised

**PANTHER**: Protein Analysis Through Evolutionary Relationships

**PARVA**: Parvin Alpha Gene

**PCC**: Posterior Cingulate Cortex

**PET**: Positron Emission Tomography

**PFC**: Prefrontal Cortex

**Pre-SMA**: Pre-Supplementary Motor Area

**PTPRD**: Protein Tyrosine Phosphatase Receptor Type D Gene

**QTLs**: Quantitative Trait Loci

**RD**: radial diffusivity

**REEP3**: Receptor Expression-Enhancing Protein 3 Gene

**ROIs**: Regions of Interest

**RSPO4**: R-Spondin 4 Gene

**SDQ**: Strengths and Difficulties Questionnaire

**SERT**: Serotonin Transporter

**SERTPR**: Serotonin Transporter Promoter Polymorphism

**SETD3**: SET Domain Containing 3, Actin Histidine Methyltransferase Gene

**SFC**: Stepwise Functional Connectivity

**SLC1A1**: Solute Carrier Family 1 Member 1 Gene

**SMA**: Supplementary Motor Area

**SN**: Salience Network

**SNPs**: Single-Nucleotide Polymorphisms

**SPECT**: Single-Photon Emission Computed Tomography

**SPM**: Statistical Parametric Mapping

**SPSS**: Statistical Package for the Social Sciences

**SRIs**: Serotonin Reuptake Inhibitors

**SSRIs**: Selective Serotonin Reuptake Inhibitors

**STN**: Subthalamic Nucleus

**TESC**: Tescalcin Gene

**TFCE**: Threshold-Free Cluster Enhancement

**VBM**: Voxel-Based Morphometry

**vlPFC**: Ventrolateral Prefrontal Cortex

**vmPFC**: Ventromedial Prefrontal Cortex

**WHO**: World Health Organization

**WM**: White Matter

**Y-BOCS**: Yale-Brown Obsessive-Compulsive Scale

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# Introduction

# 1. Introduction

## 1.1 Epidemiology and clinical characteristics of Obsessive-Compulsive Disorder (OCD)

Obsessive-Compulsive Disorder (OCD) is a distressing mental health disorder characterized by the presence of anxiety-provoking intrusive thoughts (obsessions) and ritualized and repetitive behaviors that the individual feels driven to perform to reduce the anxiety (compulsions) (American Psychiatric Association, 2013). In 1990, the World Health Organization (WHO) ranked OCD among the top ten causes of medical disability worldwide, in terms of loss of earning and diminished life quality (Murray et al., 1996). Since the 90s, there have been significant advances in terms of awareness, understanding and treatment of OCD. Nevertheless, in a new 2017 report, the WHO listed anxiety disorders, including OCD, as the sixth largest factor contributing to non-fatal health loss globally, which allows glimpses of the profound burden that OCD still imposes nowadays at both psychosocial and economic levels (World Health Organization, 2017).

Currently, OCD is estimated to affect approximately 1-3% of the general population (Mathes, Morabito and Schmidt, 2019). Although prevalence estimates of OCD are consistent across cultures, the content and themes that predominate in individuals with OCD are partly shaped by cultural, ethnic and religious experiences (Matsunaga and Seedat, 2007; Nicolini et al., 2017). The age of onset of the disorder follows a bimodal distribution, with a first peak in preadolescence (around age 10) and a second peak in early adulthood (around ages 19-20). Importantly, age-of-onset curves differ significantly between sexes, with males making up most early-onset cases, whereas females have the highest slope of cases during adolescence (Geller, 2006; Ruscio et al., 2010).

OCD is phenomenologically heterogeneous, with patients presenting wide variations in symptoms, severity, age of onset and comorbidities. Thus, the classification of OCD in

diagnostic manuals has been a subject of controversy over the last years. Traditionally, it was categorized as an anxiety disorder mainly because the anxiety raised from obsessions was considered a core feature of OCD. Moreover, OCD and anxiety disorders have significant familial aggregation, respond to similar psychotherapeutic strategies and tend to co-occur (Stein et al., 2010). Nevertheless, OCD has been shown to share significantly more similarities with other obsessive-compulsive spectrum disorders in terms of phenomenology, comorbidity, familial and genetic features, brain circuitry and treatment response than with anxiety disorders (Stein et al., 2010; Phillips et al., 2010; Hollander, et al., 2009). These findings led experts to remove OCD form the anxiety disorders section in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) (American Psychiatric Association, 2013) and to place it in a new chapter entitled 'Obsessive-Compulsive and Related Disorders' along with Body Dysmorphic Disorder, Trichotillomania, Excoriation (i.e. Skin Picking) Disorder and Hoarding Disorder, considering the latter as a new diagnostic entity, independent of the hoarding symptoms occasionally observed in OCD.

## 1.2 Etiology of OCD

Possibly due to its heterogeneity, no current theoretical model appears capable of explaining the complex etiology of OCD. Nevertheless, over the last decades, outstanding developments in the study of its neuropsychological and neurobiological bases have resulted in etiologic models that allowed for a broader understanding of the disorder both from a clinical and neurobiological perspective.

From the **cognitive-behavioral perspective**, two main theories have been proposed to account for the emergence of obsessions and compulsions: the metacognitive theory and the maladaptive habits theory. On the one hand, the classical metacognitive account postulates compulsions as goal-directed behaviors, driven by irrational thoughts and cognitive biases that the patients tend to overestimate (Myers and Wells, 2005; Robbins, Vaghi and Banca, 2019). Within this framework, individuals with OCD experience a metacognitive impairment that lead them to overestimate the importance and credibility of intrusive thoughts. Consequently, they feel increased anxiety and

discomfort that trigger the use of compulsions and ritualistic behaviors to cope with both the obsessive thoughts and their negative consequences. However, the temporal dissipation of these thoughts, together with the anxiety reduction that individuals with OCD experience after engaging in compulsive strategies, ultimately reinforce both compulsions and the maladaptive beliefs towards obsessions. On the other hand, the theory of maladaptive habits has become a prevalent model of compulsivity across many mental health disorders including eating disorders, addiction and OCD (Gillan et al., 2016). In the case of OCD, this theory flips the cause-effect direction between obsessions and compulsions proposed by the metacognitive theory, and suggests that compulsions arise from excessive habit formation while obsessions emerge as a way to explain the compulsive behavior (Gillan and Robbins, 2014; Gillan et al., 2016). Some studies have linked the automatic and inflexible behavior observed in OCD to a shift in balance away from goal-directed control and toward habits (Gillan and Robbins, 2014). Nevertheless, researchers have struggled to determine whether compulsions in OCD result from goal-directed impairments or excessive habit formation, especially because both theories exhibit neurobiological convergence with the known pathophysiology of OCD. Only in recent years, computational studies have been able to discern that habit biases in OCD are most likely a consequence of failures in goal-directed control over action (Gillan et al., 2014; Voon et al., 2014). Likewise, a subsequent task-based functional imaging study reported that the excessive habit formation in participants with OCD was linked to dysfunctions in regions that underly goal-directed control (i.e. caudate nucleus and medial orbitofrontal cortex (OFC)) (Gillan et al., 2015), which further supports the metacognitive theory.

From the **neurobiological perspective**, great advances have been made in the understanding of the anatomical, functional, neurotransmitter and genetic alterations linked to OCD. Structural and, especially, functional magnetic resonance imaging (MRI) or neuroimaging studies of OCD have been remarkably consistent in their findings compared to other neuropsychiatric disorders. Taken together, these studies support the **dysfunction of the cortico-striato-thalamo-cortical (CSTC) circuit** as the neuroanatomic substrate for obsessive-compulsive behavior, involving the basal ganglia, the thalamus and the frontal cortex (Saxena and Rauch, 2000; Pauls et al., 2014).

In the CSTC circuit, the basal ganglia are known to provide inhibition to the thalamus. This tone is regulated in two ways: by the direct and the indirect pathways, that act as accelerator and brake, respectively. More specifically, the direct pathway consists of a glutamatergic excitatory projection from the cortex to the striatum, followed by two GABAergic inhibitory projections from the striatum to the internal globus pallidus and the substantia nigra pars reticulata. Consequently, if the direct pathway is promoted, the thalamic inhibition decreases, which results in the thalamus sending glutamatergic excitatory inputs to the cortex that, in turn, projects back to the striatum, creating a positive feedback system that reinforces the selection of learned and automated sequences of behavior. The indirect pathway shares the excitatory projection from the cortex to the striatum but, from there, inhibitory inputs are sent to the external globus pallidus which, in turn, inhibits the subthalamic nucleus that, subsequently, activates the internal globus pallidus. Hence, if the indirect pathway is promoted, the thalamic inhibition will be strengthened and that will reduce the excitatory inputs from the thalamus to the cortex and from there to the striatum, causing a negative feedback loop that reduces the selection of previously learned behaviors, allowing the individual to adopt new and more adaptive ones (see **Figure 1**). The direct and indirect pathways are well balanced in health but are thought to be disrupted in OCD. In this line, OCD symptoms could be partially explained by a persistent activation of the direct pathway of the CSTC circuit, caused by the deregulation of the basal ganglia. This imbalance may lead to the hyper-activation of the circuit and, consequently, to the pathological selection of repetitive sequences of behavior and the impairment in inhibiting or replacing them with more adaptive actions (Saxena and Rauch, 2000; Pauls et al., 2014).

Importantly, the CSTC circuit is functionally segregated in loops that regulate different sequences of behavior. For instance, the **sensorimotor loop** mediates automatic responses and the transition from goal-directed to habitual behaviors by means of the connection between the premotor cortex and the supplementary motor area (SMA), the posterior putamen and the thalamus. Conversely, the cognitive loop is divided into the dorsal and ventral cognitive paths. The **dorsal cognitive loop** mediates executive functions such as working memory and planning by linking the dorsolateral and dorsomedial prefrontal cortices (dlPFC and dmPFC) and the pre-SMA with the dorsal

striatum and the thalamus, whereas the **ventral cognitive loop** is involved in motor preparation and response inhibition, connecting the inferior frontal gyrus (IFG) and the ventrolateral PFC (vlPFC) with the ventral striatum and the thalamus (Jahanshahi et al., 2015; van den Heuvel et al., 2016; Simpson et al., 2020). Finally, the **limbic loop** has classically been associated with emotion and affective processing by connecting the ventromedial PFC (vmPFC) and the anterior cingulate cortex (ACC) with the nucleus accumbens and the thalamus (Milad and Rauch, 2012) (see **Figure 2**). Hence, although OCD has been consistently associated with abnormalities in the CSTC circuit, it is important to note that different symptoms may be underpinned by dysfunctions involving distinct areas within this circuit.

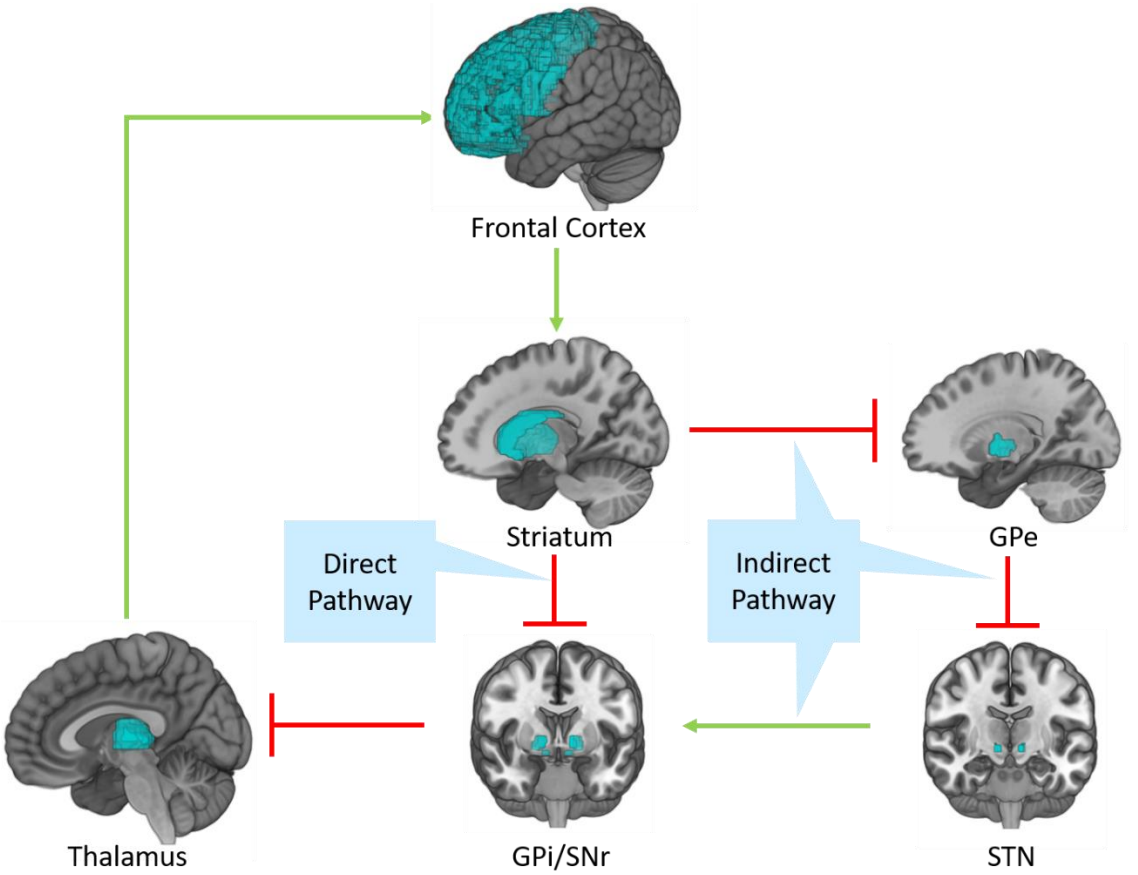


**Figure 1.** Scheme of the Cortico-Striato-Thalamo-Cortical circuitry. Green arrows represent glutamatergic excitatory pathways and red lines represent GABAergic inhibitory pathways. GPe: External Globus Pallidus; GPi: Internal Globus Pallidus; SNr: Substantia Nigra Pars Reticulata; STN: Subthalamic Nucleus. Figure adapted from Kalra and Swedo (2009) and Pauls et al. (2014).

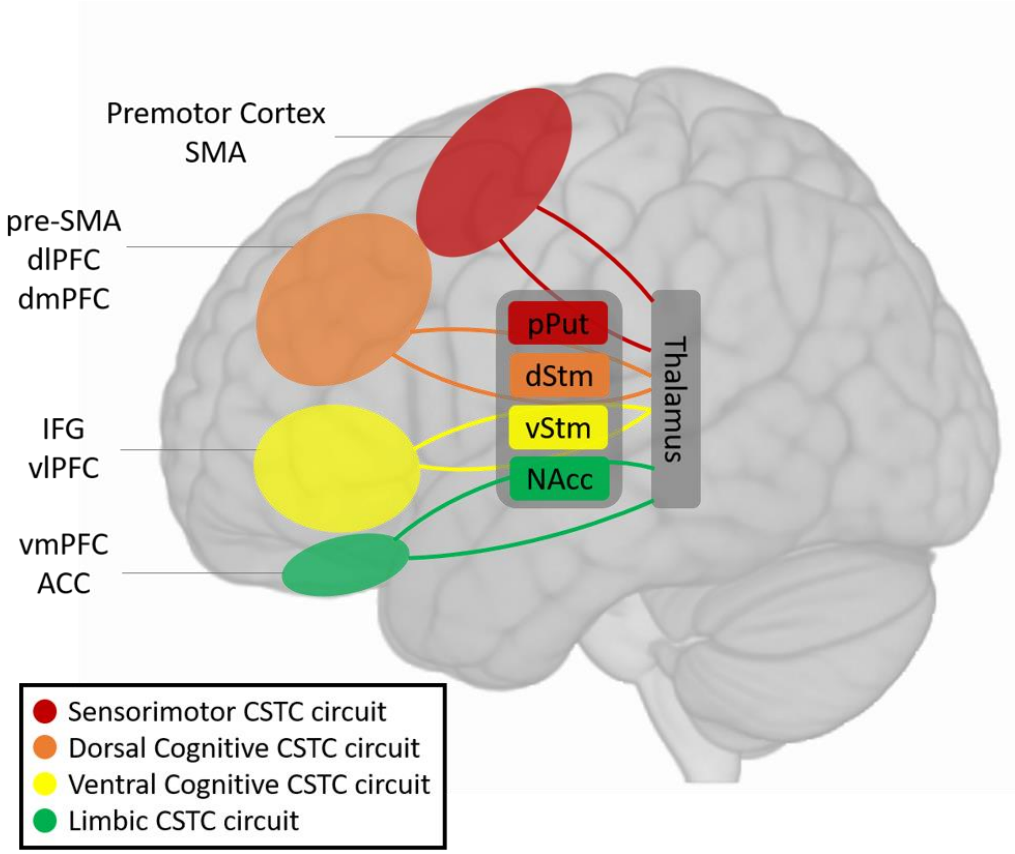


**Figure 2.** Scheme of the distinct loops of the Cortico-Striato-Thalamo-Cortical circuitry. ACC: Anterior Cingulate Cortex; CSTC: Cortico-Striato-Thalamo-Cortical; dlPFC: Dorsolateral Prefrontal Cortex; dmPFC: Dorsomedial Prefrontal Cortex; dStm: Dorsal Striatum; IFG: Inferior Frontal Gyrus; NAcc: Nucleus Accumbens; pPut: Posterior Putamen; pre-SMA: pre-Supplementary Motor Area; SMA: Supplementary Motor Area; vlPFC: Ventrolateral Prefrontal Cortex; vmPFC: Ventromedial Prefrontal Cortex; vStm: Ventral Striatum. Figure adapted from van den Heuvel et al. (2016) and Stein et al. (2019).

Moreover, imaging studies have also implicated regions classically outside the CSTC circuit in OCD, with limbic (i.e. hippocampus and basolateral amygdala) and posterior brain (i.e. parietal, occipital and cerebellar cortices) regions being the more consistently replicated across studies (Hazari, Narayanaswamy and Venkatasubramanian, 2019). These findings have been integrated into additional models such as the **triple network model**, which had been previously associated with several psychiatric disorders (Menon, 2011). The triple network model is based on aberrant functional organization within and between the salience network (SN), the frontoparietal network (FPN) and the default mode network (DMN). Importantly, the SN integrates sensory, cognitive and emotional inputs and acts as a mediator between the DMN and the FPN to balance internal mental

processes and external stimulus-driven cognitive and affective processes (Menon, 2011). In the case of OCD, the triple network model accounts for the classic frontostriatal dysfunction but also for impairments in limbic and parietal areas. This model received support from a recent meta-analysis of 18 seed-based resting state studies that reported hypoconnectivity within and between the SN, the FPN and the DMN in OCD patients (Gürsel et al., 2018). Besides, another proposal for the neurobiological mechanisms underlying OCD is the **deactivation of occipital/parietal areas involved in visual-perceptual processing** which concords with impairments in these areas (Gonçalves et al., 2010; Moreira, et al., 2019). The role of the cerebellum in the pathophysiology of OCD remains unclear, however this region has reciprocal connections with CSTC regions and exhibits abnormal functional connectivity with the DMN (Moreira, et al., 2019).

Since CSTC circuit abnormalities are still the prevailing neuroanatomic model of OCD, researchers hypothesized that the **dysregulation of the neurotransmitters** within this circuit (i.e. serotonin, dopamine, glutamate and ɣ-aminobutyric acid (GABA)) may play a role in the pathophysiology of OCD. In fact, pharmacological studies have shown that most patients with OCD respond to serotonin reuptake inhibitors (SRIs) that increase the intrasynaptic serotonin levels. In a part of non-responsive patients, SRIs can be successfully augmented with dopamine receptor antagonists, analogs of GABA or glutamate modulating drugs. However, the involvement of these neurotransmitters in effective pharmacological approaches provides insufficient evidence of their role in the pathophysiology of OCD (Graat, Figee and Denys, 2017). For instance, impaired neurotransmission could be directly contributing to OCD or could act in an indirect manner, compensating or mitigating disturbances in other neurotransmission systems. Fortunately, imaging and genetic studies have yielded more evidence of the role of these neurotransmitters on the pathophysiology of OCD. For instance, Positron Emission Tomography (PET) and Single-Photon Emission Computed Tomography (SPECT) binding studies have shown that OCD is associated with reduced presynaptic serotonin transporter (SERT) availability in limbic and paralimbic areas, the nucleus accumbens (Hesse et al., 2011), the midbrain-pons (Stengler-Wenzke et al., 2004; Hesse et al., 2005; Hasselbalch et al., 2007, Zitterl et al., 2007), and thalamic/hypothalamic regions (Hesse et al., 2005; Zitterl et al., 2007; Hesse et al., 2011), which correlated with symptom

severity, and to increased postsynaptic serotonin 2A receptor (5-HT2A) availability in cortical areas, mainly in the OFC and dlPFC, which also correlated with severity (Perani et al., 2008). These findings reflect lower synaptic serotonin levels in OCD, which seems to lead to compensatory down-regulations of presynaptic SERT, and up-regulations of 5-HT2A to increase serotonin sensitivity (Graat, Figee and Denys, 2017). Furthermore, genetic studies have found associations between SERT polymorphisms and OCD, which indicates that it might have a primary role in OCD pathophysiology (Taylor, 2012; 2015). Regarding dopamine, PET and SPECT studies have found increased presynaptic dopamine transporter (DAT) availability and reduced postsynaptic density of dopamine receptors (D1 and D2/3) in the striatum (Kim et al., 2003; Van der Wee et al., 2004; Denys et al., 2004a; Perani et al., 2008; Denys et al., 2013). Unlike serotonin, these findings indicate higher synaptic dopamine levels in OCD, which leads to compensatory up-regulations of presynaptic DAT and down-regulation of postsynaptic D1 and D2/3. However, most genetic studies have not reported associations between dopaminergic genes polymorphisms and OCD, which suggests that dopamine might not play a direct role in OCD pathophysiology (Graat, Figee and Denys, 2017). In this vein, a PET study found that the dopaminergic hyperactivity in the striatum was linked to serotonergic deficits in patients with Tourette syndrome and comorbid OCD (Wong et al., 2008). Moreover, while serotonin has been linked to OCD severity, dopamine seems to be related to symptom improvement in response to SRIs pharmacotherapy (Perani et al., 2008; Wong et al., 2008). Therefore, dopaminergic alterations might be a result of serotonergic deficits and, consequently, have a secondary role in the pathophysiology of OCD. Regarding glutamate, magnetic resonance spectroscopy (MRS) studies have shown increased glutamatergic function in the striatum (Rosenberg et al., 2000; Naaijen et al., 2015) that normalized after selective SRIs (SSRIs) treatment (Rosenberg et al., 2000). Similarly, elevated glutamate concentration has been observed in the cerebrospinal fluid (CSF) of patients with OCD (Chakrabarty et al., 2005). Apart from being an effective co-adjuvant in the pharmacotherapy of OCD, glutamate also seems to play a critical role in effective neurosurgical treatments for OCD. For instance, capsulotomy targeted at the internal capsule improves treatment-refractory OCD by lesioning glutamatergic neurons that project from the OFC to the striatum. The same mechanism has been observed in deep-brain stimulation targeting the internal capsule

of rats (McCracken and Grace, 2007; Yan et al., 2013). Congruently, decreased GABA levels have been observed in the plasma and in the medial PFC of patients with OCD (Simpson et al., 2012). Dopamine and serotonin receptors have a modulatory influence on the activity of glutamate and GABA in the CSTC circuit, and vice versa (Graat, Figee and Denys, 2017). However, genetic studies have consistently associated the glutamate transporter gene SCL1A1 with OCD, which indicates that this glutamate transporter might play a direct role in the pathophysiology of OCD (Fernandez, Leckman and Pittenger, 2018). Conversely, limited studies have assessed the association between OCD and GABA-related genes, hence, it remains unclear whether GABA alterations may be a direct or an indirect factor of the pathophysiology of OCD (Graat, Figee and Denys, 2017). Reviewing all these findings, Graat, Figee and Denys (2017) concluded that the neurotransmitter model of OCD consists of **increased glutamatergic and dopaminergic neurotransmission within frontostriatal pathways, along with decreased serotonergic and GABAergic neurotransmission in frontolimbic pathways**. These imbalances may explain increased frontostriatal activity and impaired emotion regulation, underpinned by frontolimbic areas.

In parallel, computational neuroscience studies have proposed a new model of OCD pathophysiology based on glutamate hyperactivity. More specifically, integrate-and-fire neuronal network simulation models have shown that **overactivity in glutamatergic excitatory synapses might increase the attractor properties of certain brain nodes and, consequently, lead to the over-stability of certain network states** (Rolls, Loh and Deco, 2008; Rolls, 2012). These brain nodes have been conceptualized as '**attractors**': areas with increased pulling properties that cause the dynamic functional connectivity to repeatedly converge into them (Diez and Sepulcre, 2018). In this context, the brain would get locked in over-stable cognitive states and the presence of neuronal spiking-related and potentially other noise may be insufficient to help the system move out of such state, which could lead to obsessive thoughts and, thus, to compulsive behavior (Rolls, Loh and Deco, 2008; Rolls, 2012). Interestingly, the increased excitatory activity and the consequent stability of attractor networks could occur in different brain areas causing distinct symptoms, which is congruent with the clinical heterogeneity of OCD

and with the idea that different symptoms may arise from distinct neurobiological alterations (Rolls, Loh and Deco, 2008).

**Heritability and genetic studies** have also contributed to a better understanding of the pathophysiology of OCD. Familial aggregation studies have shown that OCD is familial, with first degree relatives of patients with OCD having a rate of 10.9% of suffering the disorder, compared to 1.9% in subjects with no relatives affected. Twin studies have reported a concordance of 65-87% in monozygotic twins and of 25-47% in dizygotic twins, which demonstrates that the familiarity is partially due to the contribution of genetic factors (Vallejo and Berrios, 2006).

According to subsequent genetic linkage, candidate gene, and genome-wide association studies (GWAS), OCD appears to be a complex polygenetic condition. The most consistent finding of **linkage and candidate gene studies** has been the possible contribution of the SLC1A1 gene, located on chromosome 9 (Fernandez, Leckman and Pittenger, 2018). Importantly, this gene encodes the neuronal glutamate transporter EAAT3, hence, this finding provides support to the glutamate hypothesis. Moreover, a remarkable study, reporting the results of two meta-analysis, found that OCD was associated with serotonin-related genes (i.e. 5-HTTLPR/SLC6A4 and HTR2A) and, at a trend level, with dopamine-related genes (DAT1/SLC6A3 and DRD3) and one glutamate-related gene (SLC1A1) (Taylor, 2012). Notably, candidate gene studies included in these meta-analyses were designed according to the current knowledge of neurotransmitters involved in OCD, hence, dopamine-related and glutamate-related genes were included in much less studies that serotonin-related genes, which may explain why they did not reach statistical significance (Taylor, 2012).

However, linkage and candidate gene studies fail to detect genes with smaller effects or copy-number variants, which can be assessed by GWAS. In this line, **GWAS** performed by the International OCD Foundation Genetics Collaborative (IOCDF-GC) and the OCD Collaborative Genetics Association Study (OCGAS), each one including around 1,400 patients with OCD, found no single-nucleotide polymorphisms (SNPs) reaching genome-wide significance. However, the first one detected an implication of methylation quantitative trait loci (QTLs) and frontal lobe expression QTLs (eQTLs) (Stewart et al., 2013), whereas the second one, suggested an involvement of the PTPRD gene, which

promotes glutamate receptor differentiation at a presynaptic level (Mattheisen, et al., 2015). The first genome-wide significant association in OCD was reported by Noh et al. (2017) and involved the NRXN1 gene, which encodes the synapse cell-adhesion protein neurexin1α. Moreover, they found three additional genes with a strong association with OCD: HTR2A, which encodes the 5-HT2A serotonin receptor, consistently involved with the SSRIs pharmacotherapy in OCD; CTTNBP2, involved in synapse maintenance; and REEP3, involved in vesicle trafficking. However, this study did not used a GWAS approach, instead the authors recruited 592 patients with OCD and analyzed sequencing data for 608 OCD candidate genes, retrieved from animal models and human studies, and prioritized variants based on functional and conservation annotations. Hence, albeit the four genes reported are involved in the neural circuits linked to OCD, including serotonin and glutamate signaling, synaptic function and the CSTC circuit, the results should be interpreted with caution.

A recent consortium meta-analysis assessing 2,688 patients with OCD located variants associated with OCD in or near the genes ASB13, RSPO4, DLGAP1, PTPRD, GRIK2, FAIM2 and CDH20 (IOCDF-GC and OCGAS, 2018). It is worth noting that, among them, DLGAP1 and GRIK2 are glutamate-related genes. Remarkably, the glutamate hypothesis has also received support from a recent review that assessed the interactome of 151 genes that had been previously linked to OCD and found that GRIN2A, GRIN2B, which encode glutamate N-Methyl-D-Aspartate (NMDA) receptor subunits, and GRIA2, which encodes a glutamate α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid (AMPA) receptor subunit, were the most central nodes of the network. Moreover, functional enrichment analysis revealed that glutamate-related pathways were the main deficient system in patients with OCD (Bozorgmehr, Ghadirivasfi and Shahsavand-Ananloo, 2017).

Overall, findings from genetic studies have been consistent with the CSTC model of OCD and support the proposal of glutamate/GABA imbalance within this circuit. However, they have also reflected the polygenic and heterogeneous etiology of the disorder.

Although the etiology of OCD remains unclear, studies assessing its pathophysiology have greatly contributed to the understanding of the cognitive and neurobiological bases of the disorder. Moreover, these studies have set the framework for future research, giving a central role to the clinical heterogeneity in OCD.

## 1.3 Symptom heterogeneity in OCD: the multidimensional model

Although OCD is considered a unitary nosological entity in diagnostic manuals like DSM-V, this disorder is characterized by remarkably heterogeneous symptoms, to the extent that two patients with an OCD diagnosis may exhibit completely different, nonoverlapping symptom patterns (Mataix-Cols, Rosario-Campos and Leckman, 2005). The **clinical heterogeneity** of OCD poses difficulties not only in the diagnosis and therapeutic strategies, but also on how researchers approach the disorder. Studies assessing OCD as a single entity have provided relatively heterogeneous neurobiological correlates, leading to the hypothesis that different clinical manifestations of OCD may be reflecting underlying neurobiological differences. Hence, considering the clinical variability in research settings could serve as a tool to define more homogeneous subgroups of patients and may aid in the identification of more robust endophenotypes (Mataix-Cols, Rosario-Campos and Leckman, 2005).

In this vein, the **multidimensional model** accounts for the phenotypic heterogeneity by categorizing symptoms into distinct clinical dimensions. These dimensions can be understood as a spectrum of potentially overlapping syndromes that may coexist in any patient and be continuous with usual obsessive-compulsive phenomena or extend beyond the traditional boundaries of OCD (Mataix-Cols, Rosario-Campos and Leckman, 2005). Over the last 30 years, multiple studies have attempted to define dimensions that explain a large portion of the clinical variance through factorial analyses. The first contribution to the multidimensional model of OCD was that of Baer (1994). He factor-analyzed OCD symptoms and identified three factors accounting for 48% of the variance, which were named *symmetry/hoarding*, *contamination/cleaning*, and *pure obsessions* (Baer, 1994). Following studies, using different factorial approaches and also different instruments in the assessment of symptoms, have obtained remarkably similar results: they consistently showed that more than three factors explained more than 60% of the variance and that the most consistent factorial solutions were those of four or five dimensions (Mataix-Cols, Rosario-Campos and Leckman, 2005). A subsequent meta-analysis examined the data from 21 studies including 5124 participants (4 of them

involving 679 children participants) and obtained the following four factors: (1) *symmetry/ordering*, which contained symmetry obsessions and repeating, ordering, and counting compulsions; (2) *contamination/cleaning*, which grouped contamination obsessions and cleaning compulsions; (3) *hoarding*, which contained obsessions around the idea of needing objects in the future and hoarding compulsions; and (4) *forbidden thoughts*, which included aggression, sexual, religious, and somatic obsessions and checking compulsions (Bloch et al., 2008). They performed an additional stratified meta-analysis to determine whether the factor structure changed between adult and pediatric samples. The factor structure of studies involving adults was identical to the one obtained with the total sample. Only a few differences were observed in the case of children: checking compulsions shifted from the *forbidden thoughts* to the *symmetry/ordering* factor and somatic obsessions shifted from the *forbidden thoughts* to the *contamination/cleaning* factor (Bloch et al., 2008). After this meta-analysis, additional studies have replicated the same factor structure of OCD symptoms in pediatric samples, validating the multidimensional model in children and adolescents (Stewart et al., 2008; Nikolajsen, Nissen and Thomsen, 2011). Importantly, three symptom dimensions have been consistent across these studies and include *symmetry/ordering* (also known as *ordering*), *contamination/cleaning* (also known as *washing*), and *hoarding*. However, the fourth dimension, *forbidden thoughts*, has varied. To clarify these inconsistencies, more recent attempts have performed more refined factor analyses (i.e. studying questionnaires at an item-level instead of using predefined symptom categories) that have resulted in five symptom dimensions for OCD. In these cases, the symptoms previously classified as *forbidden thoughts* were grouped into two new dimensions: *doubting/checking* (also known as *aggressive/checking*), which includes obsessions around doubt or fear of causing unintentional harm and checking compulsions; and *unacceptable/taboo thoughts* (also known as *sexual/religious* or *obsessing*) which includes a broad set of obsessions of violent, sexual and religious content (Brakoulias et al., 2013).

Nevertheless, some OCD symptoms such as counting compulsions and somatic obsessions are still not well accounted for in the latter five-dimension proposal (Brakoulias et al., 2013). Although counting compulsions have been associated with

*symmetry/ordering* in some studies (Cullen et al.,2007; Hasler et al., 2005; 2007; Mataix-Cols et al., 1999), this association is not consistent enough (Mataix-Cols et al., 2002; Matsunaga et al., 2008; Brakoulias et al., 2013). Consequently, in some factor analyses and dimensional instruments, such as the Obsessive-Compulsive Inventory (OCI), its revised version (OCI-R) and the OCI-Child Version (OCI-CV), counting compulsions were grouped into a new dimension named *neutralizing* (Foa et al., 1998; 2002; 2010). On the other hand, the classification of somatic obsessions has provided mixed findings, loading either with *doubting/checking* (Baer, 1994; Denys et al., 2004b; Hasler et al., 2005), with *unacceptable/taboo thoughts* (Mataix-Cols et al., 2002; Kim, Lee and Kim, 2005; Cullen et al., 2007; Albert et al., 2010), or directly forming a separate dimension named *somatic/hypochondriacal* (Stein, Andersen and Overo, 2007; Stein et al., 2008). Further research is warranted in order to clarify this variability in classification schemes.

Although the multidimensional model of OCD has some caveats, it has created an interesting framework to advance in the understanding of OCD endophenotypes both in adult and pediatric samples. Furthermore, it has led to the development of several psychometrically validated clinician-rated, patient-rated, parent-rated and child-rated measures to assess OCD from a dimensional perspective. However, these measures differ in some of the dimensions assessed, the rater and the adjunctive measures included (i.e. impairment level, family accommodation and insight), which inevitably hampers the comparison of findings obtained with different instruments. Nevertheless, the use of the multidimensional model of OCD has provided a significant body of research describing sociodemographic, neuropsychological, clinical, and genetic differences among dimensions, albeit results are far from being consistent across studies. Additionally, researchers have assessed the modulatory effect of dimensions on the neural correlates of OCD, which will be described in detail in the "Summary of previous research on the structural and resting-state functional connectivity correlates of OCD" section (see below).

Regarding **sociodemographic differences**, *contamination/cleaning* and *doubting/checking* symptoms are more prevalent in females than in males, and tend to occur during and after menarche and/or pregnancy, which suggest that ovarian hormones might play a role in the pathogenesis of these symptoms. Conversely,

*symmetry/ordering, unacceptable/taboo thoughts* and *neutralizing* dimensions are more frequent in males than in females (Labad et al., 2008; Russel, Fawcett and Mazmanian, 2013; Torresan et al., 2013; Cherian et al., 2014; Mathes, Morabito and Schmidt, 2019). Nevertheless, some studies did not find between-sex differences across symptom dimensions (Politis et al., 2017; Raines et al., 2018). Interestingly, Raines et al. (2018) reported that correlations between OCD symptom dimensions were stronger in males than in females. This might lead to males exhibiting symptoms from different dimensions, whereas females may tend to present symptoms within a specific dimension, which would have a significant implication in treatment strategies. A possible explanation for this finding could be the earlier age of onset observed in males compared to females (Ruscio et al., 2010). Since males tend to develop OCD earlier, this might result in a broader presentation of the disorder (Raines et al., 2018). In turn, research into the age of onset has consistently shown that *symmetry/ordering* symptoms tend to develop earlier than other OCD dimensions. Furthermore, *symmetry/ordering* and *unacceptable/taboo thoughts* dimensions are more prevalent in individuals with early OCD onset (Uguz et al., 2006; Labad et al., 2008; Kichuk et al., 2013).

Literature assessing **psychosocial risk factors** for OCD dimensions has reported very interesting findings. *Symmetry/ordering* and *unacceptable/taboo thoughts* dimensions have been linked to perinatal insults, whereas the *doubting/checking* dimension has been associated with poor childhood motor skills and the loss of a parental figure (Grisham et al., 2011). On the other hand, the *hoarding* dimension has been associated with low parental emotional warmth (Alonso et al., 2004), and with perceived poor maternal care, maternal overprotection and maternal overcontrol in women with OCD (Chen et al., 2017). Finally, patients with OCD in which the onset of the disorder could be linked to a stressful life event exhibited higher prevalence of *contamination/cleaning* and *symmetry/ordering* dimensions and *somatic obsessions* (Real et al., 2011; Rosso et al., 2012). Notably, the relationship between the *symmetry/ordering* dimension and stressful life events contradicts the notion that these symptoms are linked to neurodevelopmental insults and earlier OCD onset.

At the neuropsychological level, OCD dimensions have been associated with distinct **dysfunctional beliefs and cognitions**. For instance, Wheaton et al. (2010) reported that d*oubting/checking* and *contamination/cleaning* dimensions were linked to increased responsibility and threat estimation beliefs. Conversely, *unacceptable/taboo thoughts* were associated with increased importance and control of thoughts, and *symmetry/ordering* with increased perfectionism and intolerance to uncertainty. However, the *hoarding* dimension was not assessed. Remarkably, Brakoulias et al. (2014) replicated most of these findings except for the association between *contamination/cleaning* and increased responsibility and threat estimation beliefs. The authors hypothesized that this could be related to the limited range of cognitions assessed by the Obsessive-Beliefs Questionnaire (OBQ). Similarly, the *hoarding* dimension was not associated with any of the three domains of the OBQ (i.e. responsibility/threat overestimation, importance/control of thoughts and perfectionism/certainty), which supports the division between Hoarding disorder and OCD. However, it is possible that individuals with OCD and predominant *contamination/cleaning* or *hoarding* symptoms show dysfunctional beliefs and cognitions that are not assessed by the OBQ. For instance, individuals with OCD and *contamination/cleaning* symptoms tend to exhibit disgust-specific cognitions (Olatunji et al., 2019), whereas individuals with OCD and *hoarding* symptoms may present cognitions related to an overestimation of the importance of objects or to the need to accumulate them for future use (Starcevic et al., 2011). Taken together, these findings suggest that different compulsions may be motivated by distinct dysfunctional beliefs and cognitions, which further supports the need of a multidimensional model.

Continuing with neuropsychological differences, numerous studies have assessed **cognitive performance** across symptom dimensions. In this line, a recent review of 23 studies reported the following differences between dimensions: patients with *doubting/checking* symptoms exhibited more cognitive impairments compared to other dimensions. These include poorer performance compared to patients with *contamination/cleaning* on planning, cognitive control, set shifting, as well as general, verbal and non-verbal memory, and negative priming. Additionally, patients with *doubting/checking* symptoms also underperformed on working memory compared to

patients with predominant *obsessing, contamination/cleaning*, and *mixed* symptoms (Cameron et al., 2020). These results are congruent with a previous meta-analysis, in which patients with *contamination/cleaning* symptoms showed better task performance than patients with *doubting/checking* symptoms in 8 out of 10 cognitive domains (Leopold and Backenstrass, 2015). Taken together, these findings support the notion that harm-avoidance symptoms might be linked to an attentional bias directed toward fear-based stimuli, leading to executive function impairments, while also causing anxiety-like impairments, such as poor memory and decision making. In contrast, in another recent meta-analysis, the *symmetry/ordering* dimension was more strongly related to poorer neuropsychological performance overall, and in attention, visuospatial ability, and verbal working memory compared to the *obsessing/checking* dimension. (Bragdon, Gibb and Coles, 2018). However, this could be partially explained by the fact that *doubting/checking* and *obsessing* symptoms were merged in the *obsessing/checking* dimension, which may have overshadowed the cognitive differences between them and, thus, hinder the comparison with *symmetry/ordering.* Remarkably, none of the previously mentioned meta-analyses assessed *hoarding* symptoms, mainly because many researchers viewed them as a distinct syndrome, even prior to its definition in the DSM-V. In earlier studies, the *hoarding* dimension was associated with greater procedural learning and decision-making impairment (Lawrence et al., 2006; Goldman et al., 2008). Moreover, when compared to patients with OCD without hoarding symptoms, patients with this dimension exhibited increased global functioning impairment (Wheaton et al., 2008). In youth with OCD, *hoarding* and *symmetry/ordering* dimensions were associated with increased cognitive sequelae, whereas only the latter was linked to greater cognitive impairment in nonverbal fluency, processing speed and response inhibition and switching (McGuire et al., 2014).

At the clinical level, OCD dimensions differ in insight, treatment response and comorbidity with other psychiatric disorders. Regarding **insight**, higher severity of total obsessive-compulsive symptoms and, more specifically, *contamination/cleaning* and *hoarding* dimensions were associated with poor insight (Jakubovski et al., 2011; Cherian et al., 2012; Fontenelle et al., 2013; de Avila et al., 2019). Conversely, *aggressive obsessions* and *unacceptable/taboo thoughts*, were linked to good insight (Cherian et

al., 2012), which is congruent with the notion that patients with predominant obsessions tend to exhibit increased importance and control of these thoughts.

Focusing on **treatment response**, a meta-analysis including 21 studies and 3,039 participants reported that patients with *hoarding* symptoms were significantly less likely to respond to traditional OCD treatments, including both pharmacological and behavioral therapy (Bloch et al., 2014). Additional studies have shown that cognitive-behavioral therapy may be less effective for *unacceptable/taboo thoughts* and *hoarding* dimensions (Rufer et al., 2006; Williams et al., 2014), whereas pharmacological treatment (i.e. with SRIs, citalopram and escitalopram) may be more effective for *doubting/checking* and *unacceptable/taboo thoughts* dimensions but less effective for *symmetry/ordering, hoarding* and *contamination/cleaning*. Albeit the *unacceptable/taboo thoughts* dimension was linked with better acute pharmacological response, it has also been associated with poor long-term outcome and pharmacological treatment refractoriness (Stein, Andersen and Overo, 2007; Stein et al., 2008; Landeros-Weisenberger et al., 2010; Hazari, Narayanaswamy and Arumugham, 2016). Finally, surgical procedures such as deep-brain stimulation, cingulotomy or capsulotomy, reserved for patients with treatment-refractory OCD, seem to be effective in most OCD dimensions, except for *symmetry/ordering* and *hoarding* (Denys et al., 2010; Gong et al., 2019). Caution is warranted when interpreting these results due to the use of different treatment strategies and the typically small samples assessed in most of these studies.

Regarding **psychiatric comorbidities**, the largest multicentric study to date assessed 1,001 patients with OCD and found a high comorbidity of anxiety disorders in all dimensions, except for the *symmetry/ordering* dimension. Additionally, the *doubting/checking* dimension was associated with impulse-control and skin picking disorders; *unacceptable/taboo thoughts* correlated with mood disorders, tic disorder and body dysmorphic disorder; the *contamination/cleaning* dimension was related to hypochondriasis; and the *hoarding* dimension correlated with depressive disorders, impulse-control disorders, Attention Deficit Hyperactivity Disorder (ADHD), and tic disorder (Torres et al., 2016). Some of these findings were previously observed in studies with smaller yet considerable sample sizes, which also reported alternative associations. For instance, they linked *contamination/cleaning* with eating disorders and

*symmetry/ordering* with ADHD, alcohol dependence and bulimia (Hasler et al., 2005; 2007; Torresan et al., 2013). Studies assessing exclusively pediatric samples have reported somewhat similar results: with anxiety disorders being highly comorbid across most dimensions and *unacceptable/taboo thoughts* correlating with mood disorders (Ortiz et al., 2016; Højgaard et al., 2017; 2018; Cervin et al., 2020). Additionally, *symmetry/ordering* and *symmetry/hoarding* were associated with neurodevelopmental comorbidities and, more specifically, with ADHD, Tourette's disorder, tic disorder, oppositional defiant disorder and conduct disorder (Ortiz et al., 2016; Højgaard et al., 2018).

**Genetic studies** have found significant genetic overlap across OCD dimensions, with *hoarding* sharing the least amount of genetic liability with other dimensions (Iervolino et al., 2011). However, there seem to be relevant genetic differences among dimensions. For instance, *hoarding*, *contamination/cleaning,* and *symmetry/ordering* dimensions have shown to have a stronger familiarity than other dimensions (Viswanath, et al., 2011; Brakoulias et al., 2016). Specific genetic bases have been identified for the different OCD dimensions: *contamination/cleaning* has been associated with polymorphisms in the brain-derived neurotrophic factor gene (BDNF), 5-hydroxytryptamine receptor 3E (HTR3E), and the estrogen receptor 1 gene (ESR1) (Alonso et al., 2011; Lennertz et al., 2014; Taj et al., 2018). *Symmetry/ordering* has been linked to polymorphisms in the SERT promoter polymorphic region (SERTPR), the dopamine receptor D4 gene (DRD4), glutamate ionotropic receptor NMDA-type subunit 2B gene (GRIN2B), Importin 8 gene (IPO8) and Caprin Family Member 2 gene (CAPRIN2) (Hasler, Kazuba and Murphy, 2006; Taj et al. 2013; Kohlrausch et al., 2016; Alemany-Navarro et al., 2020). Importantly, the SERTPR gene has also been associated with *unacceptable/taboo thoughts* and *neutralizing* dimensions (Cavallini et al., 2002; Kim, Lee and Kim, 2005). *Hoarding* has been linked to a variant within neurotrophic receptor tyrosine kinase 3 gene (NTRK) (Alonso et al., 2008) and *neutralizing* has been associated with a variant in the LIM homeobox transcription factor 1 alpha gene (LMX1A) (Melo-Felippe, Fontenelle and Kohlrausch, 2019). Finally, a recent gene-based analyses revealed an association between *hoarding* and SET Domain Containing 3, Actin Histidine Methyltransferase (SETD3), which is involved in apoptotic processes and transcriptomic

changes; and between *doubting/checking* and Carboxypeptidase E (CPE), which contributes to neurotrophic functions and the synthesis of peptide hormones and neurotransmitters (Alemany-Navarro et al., 2020).

Broadly, the above-mentioned findings regarding sociodemographic, neuropsychological, clinical and genetic differences between OCD symptom dimensions have been relatively heterogeneous and lack replication. This can be explained by the different definition and characterization of dimensions across studies. First, the factorial analyses implemented in the studies yielded a variable number of symptom dimensions, which consequently causes symptoms to group in different manners. For example, symmetry concerns and ordering compulsions were sometimes grouped in a unique dimension (i.e. *symmetry/ordering)*, yet in other cases they were merged with hoarding symptoms in a *symmetry/hoarding* dimension. Second, different instruments were used across studies and, even more importantly, different approaches were implemented to assess associations. In this line, many studies classified participants according to their predominant symptom dimension, which inevitably underestimates the effect that other less prominent symptoms may have on the results. Importantly, this approach may hinder the characterization of less prevalent dimensions that would be overshadowed by dimensions that are more prevalent and predominant in samples of patients with OCD, such as *doubting/checking* and *contamination/cleaning*. To avoid that, fewer studies used a truly dimensional approach in which all symptom dimensions were quantified and, hence, the observed differences between dimensions were more reliable. In conclusion, there are important limitations hampering the comparison between these studies and limiting the generalizability of their findings; nevertheless, they provide evidence of significant differences among dimensions beyond symptom presentation, further supporting the multidimensional model of OCD. The neural correlates of symptom heterogeneity are detailed in the following section.

## 1.4 Summary of previous research on the structural and resting-state functional connectivity correlates of OCD

### Structural MRI studies assessing OCD as a homogenous clinical entity

Earlier structural MRI studies focused on detecting volumetric differences between patients with OCD and healthy controls. To do so, researchers typically implemented a traditional morphometric approach, in which the volume of the whole brain or specific regions was obtained by manually drawing regions of interest (ROIs) on the structural image of each subject and then calculating the volume enclosed. This approach was extremely time-consuming and could only assess rather large ROIs, which limited the number of both subjects and regions included in the studies and, therefore, the statistical power and the generalizability of the findings. Consequently, **voxel-based morphometry** (VBM) rapidly gained popularity within the neuroimaging community because of its important methodological advantages. VBM automatically quantifies the density of any brain tissue at the voxel-wise and whole-brain level. Therefore, it is time-efficient and allows to detect microstructural differences without relying on a priori ROIs (Ashburner and Friston, 2000). For more information on the bases of structural MRI and VBM, please see the Structural MRI section in the Methods of this thesis.

The first case-control study implementing VBM to compare brain volumes between patients with OCD and healthy controls was published in 2001 (Kim et al., 2001), and Pujol et al. published a highly influential manuscript in 2004, reporting gray matter volume increases in subcortical regions together with volume decreases in different cortical areas (Pujol et al., 2004). Since then, and specially over the last decade, multiple meta-analyses have been published, assessing the structural correlates of OCD with VBM (Radua and Mataix-Cols, 2009; Radua et al., 2010; Rotge et al., 2010; Peng et al., 2012; Eng, Sim and Chen, 2015; Hu et al., 2017; Picó-Pérez et al., 2020). The most robust findings involved volume abnormalities in areas of the fronto-striatal circuitry. In all these meta-analyses, patients with OCD exhibited increased grey matter (GM) volume in the lenticular nucleus, mainly in the putamen, and, in most cases, extending to the caudate nucleus and the thalamus (Radua and Mataix-Cols, 2009; Radua et al., 2010;

Peng et al., 2012; Eng, Sim and Chen, 2015; Hu et al., 2017; Picó-Pérez et al., 2020). Importantly, increased GM volume in the lenticular nucleus correlated with OCD symptom severity (Radua and Mataix-Cols, 2009) and was found to be exclusive of OCD, when compared to other anxiety disorders (Radua et al., 2010). Moreover, in most recent meta-analysis, age was significantly associated with increased putamen volume, which suggests that patients with OCD show volume preservation in this area with increasing age (Picó-Pérez et al., 2020). This finding is congruent with the initial study of Pujol et al. (2004) and with a multicenter mega-analysis, assessing 412 adults with OCD and 368 healthy controls from the six centers that constitute the OCD Brain Imaging Consortium (OBIC) (de Wit et al., 2014). However, this association was not reported in previous meta-analyses. For instance, Hu et al. (2017) assessed GM alterations in youths and adults with OCD and reported that increased GM volumes in the lenticular nucleus were observed in both age groups. Nevertheless, they also found that greater GM volumes in this area were more prominent in both pediatric and adult medicated patients. Further research is warranted to determine whether this abnormal increase in volume is due to disease chronicity or medication effects. Additional GM volume increases have been reported in the lateral OFC (Rotge et al., 2010), the middle frontal and cingulate gyri (Eng, Sim and Chen, 2015), the superior parietal cortex, the somatosensory cortex (Radua and Mataix-Cols, 2009; Eng, Sim and Chen, 2015; Peng et al., 2012), the culmen (Eng, Sim and Chen, 2015), the fusiform gyrus, the hippocampus (Picó-Pérez et al., 2020), and the cerebellum (de Wit et al., 2014; Hu et al., 2017; Picó-Pérez et al., 2020). However, Hu et al. (2017) reported that increases in the GM volume of the cerebellum were only observed in adult patients with OCD.

In contrast, across these meta-analyses, GM reductions have been consistently reported mainly in prefrontal regions, encompassing the ACC (Radua and Mataix-Cols, 2009; Radua et al., 2010; Peng et al., 2012; de Wit et al., 2014; Hu et al., 2017; Picó-Pérez et al., 2020), the dmPFC (Radua and Mataix-Cols, 2009; Radua et al., 2010; de Wit et al., 2014), the OFC (Rotge et al., 2010; Peng et al., 2012; Hu et al., 2017), and the IFG (de Wit et al., 2014; Hu et al., 2017; Picó-Pérez et al., 2020). To a lesser extent, OCD has also been linked to decreased GM in the dlPFC (Rotge et al., 2010; Peng et al., 2012), the vmPFC (Picó-Pérez et al., 2020) and the frontal eye fields (Peng et al., 2012). Additional

associations have been reported outside the frontal cortex. In this vein, OCD was linked with GM reductions in the insula (de Wit et al., 2014; Eng, Sim and Chen, 2015), temporal regions (Eng, Sim and Chen, 2015; Picó-Pérez et al., 2020) and the supramarginal gyrus (Rotge et al., 2010). Age and medication interactions were also observed in GM reductions associated with OCD. The OBIC mega-analysis revealed that, in patients with OCD, temporal regions exhibited a loss of volume with increasing age. Conversely, they observed volume preservation in the OFC and insula with increasing age. Since these areas have been reported to be reduced in OCD, their preservation with age might reflect a compensatory mechanism (de Wit et al., 2014). Additionally, decreased volume in the visual cortex was only observed in pediatric OCD (Hu et al., 2017). Medication interaction analyses showed that medicated youth exhibited more prominent volume reductions in the inferior and medial frontal gyri. Conversely, medicated adults had more prominent volume reductions in the IFG, the ACC and the OFC (Hu et al., 2017). Interestingly, meta-analyses assessing volumetric differences across psychiatric disorders have shown that smaller GM volumes in the dmPFC/dorsal ACC are not specific of OCD, but also present in anxiety disorders (Radua et al., 2010). Similarly, smaller GM volumes in the dorsal ACC and insula/operculum were also observed in anxiety disorders, schizophrenia, bipolar disorder, depression and addiction (Goodkind et al., 2015).

Despite the many advantages of VBM, this technique does not differentiate among distinct cortical morphological properties. Cortical GM volume abnormalities observed with VBM depend on a combination of alterations in GM thickness and surface area. Importantly, cortical GM thickness and surface area have distinct developmental trajectories: while cortical thickness consistently changes across lifespan due to development, environment and disease factors, surface area reflects earlier neurodevelopmental changes (Raznahan et al., 2011; Boedhoe et al., 2018). Hence, assessing these cortical morphometrical properties independently may allow to differentiate alterations that occurred at distinct neurodevelopmental stages (Boedhoe et al., 2018).

In this line, some recent mega-analyses have used surface-based methods to assess not only **cortical thickness and surface area**, but also **subcortical volume** in OCD. For

instance, Fouche et al. (2017), assessed cortical thickness and subcortical volume alterations in the OBIC sample previously used in the VBM mega-analysis (de Wit et al., 2014). The authors found that adults with OCD, compared to controls, exhibited decreased cortical thickness in superior and inferior frontal, precentral, posterior cingulate, middle temporal, inferior parietal and precuneus gyri, and smaller hippocampal volume. Moreover, they reported increased thinning of the parietal cortex in OCD with age (Fouche et al., 2017). Subsequent mega- and meta-analyses using this approach were bolstered by the ENIGMA-OCD Working Group. In their two studies, the authors assessed alterations in subcortical volume, cortical thickness and surface area in both pediatric and adult OCD using considerably large samples (i.e. 1,830/1,905 patients with OCD and 1,759/1,760 control subjects) (Boedhoe et al., 2017; 2018). In their first study, Boedhoe et al. (2017) assessed subcortical brain volume and found that adult patients with OCD had reduced hippocampal volume and increased pallidum volume when compared to controls. In both cases, the effect was stronger in medicated patients. The pallidum finding was more prominent in adults with early OCD onset and, since it is congruent with most previous VBM mega- and meta-analyses (Radua and Mataix, 2009; Radua et al., 2010; Peng et al., 2012; de Wit et al., 2014; Eng, Sim and Chen, 2015; Hu et al., 2017; Picó-Pérez et al., 2020), it seems to be specific of OCD. Conversely, the hippocampal result was more prominent in adults with late OCD onset and comorbid depression. This finding is congruent with the surface-based OBIC mega-analysis (Fouche et al., 2017) but contradicts a recent VBM meta-analysis (Picó-Pérez et al., 2020). However, hippocampal reductions have also been associated with other psychiatric disorders such as major depressive disorder, post-traumatic stress disorder, schizophrenia and bipolar disorder (Bromis et al., 2018; Haukvik et al., 2018), which suggests that it might be a generalized feature of psychiatric disorders related to chronic stress. Boedhoe et al. (2017) also reported that unmedicated pediatric OCD patients exhibited larger thalamic volumes than pediatric controls. This finding concurs with a previous ROI-based meta-analysis that included pediatric patients (Rotge et al., 2009) and suggests that enlarged thalamic volume might be an earlier marker of the disease potentially associated with neurodevelopmental impairments (Boedhoe et al., 2017).

In their second study, Boedhoe et al. (2018) assessed cortical thickness and surface area and found that youth with OCD showed thinner left and right inferior parietal, left superior parietal and lateral occipital cortices than age-matched controls. In contrast, adults with OCD had thinner bilateral inferior parietal cortices and lower surface area of the transverse temporal cortex than age-matched controls. The parietal thinning finding is consistent with the surface-based OBIC mega-analysis (Fouche et al., 2017), but, in this case, extended to pediatric OCD. Hence, it suggests that impaired cortical maturation in OCD may lead to a thinner parietal cortex in childhood, that persists into adulthood (Boedhoe et al., 2018). However, medication status seemed to be an important confounder in this study. Unmedicated youth and adults with OCD did not almost differ from controls in cortical thickness and surface area. In contrast, medicated youth with OCD exhibited cortical thinning of the above-mentioned parietal and occipital areas, but also showed widespread lower surface area, mainly in frontal regions, compared to controls. Similarly, medicated adults with OCD exhibited widespread cortical thinning that extended throughout the brain, compared to controls (Boedhoe et al., 2018). However, it is impossible to characterize the specific effect of medication due to the cross-sectional nature of the study and the lack of detailed information on the treatment history, duration and dosage. Nevertheless, this study showed that alterations in cortical thickness were more pronounced in medicated adults with OCD, whereas surface area abnormalities were more prominent in medicated youth with OCD, which supports the use of surface-based approaches that characterize distinct cortical morphometry properties to assess alterations occurring at different neurodevelopmental stages.

White matter (WM) alterations in OCD have been understudied in VBM. Although voxel-wise whole-brain WM density is automatically available in VBM, most studies chose not to report WM findings, which causes a reporting bias. To overcome this limitation, the OBIC VBM mega-analysis published their WM findings and reported decreased frontal WM volume in patients with OCD (de Wit et al., 2014). This reduction was more pronounced in medicated patients and in patients with comorbid major depressive disorder. However, they also observed an association between preservation of the frontal WM volume with aging in OCD. Frontal volume alterations may underlie

cognitive impairments, which have been consistently reported in OCD. In this context, the authors interpreted WM preservation with aging in OCD as a compensatory activation-induced neuroplasticity mechanism (de Wit et al., 2014).

In contrast, WM changes have been more commonly studied with **Diffusion Tensor Imaging** (DTI) approaches. This method is based on the quantification of water diffusion through the brain which provides information about the integrity and organization of WM tracts. Fractional anisotropy (FA) and mean diffusivity (MD) are the main measures of WM integrity and usually are negatively correlated, with FA being decreased and MD being increased in pathological WM (Le Bihan et al., 2001). Meta-analyses of DTI studies assessing OCD have shown WM abnormalities in the frontostriatal circuits, mainly in the ACC and the OFC, which concurs with VBM findings (de Wit et., 2014). Additionally, they found abnormal connectivity between lateral frontal and parietal regions and microstructural abnormalities in the corpus callosum (i.e. decreased connectivity in the rostrum and hyperconnectivity in the genu) and in intra-hemispheric bundles, linking prefrontal areas to posterior parietal and occipital association cortices (Piras et al., 2013; Peng et al., 2012). A more recent meta-analysis of the ENIGMA-OCD Working Group reported WM alterations in adults with OCD located in the posterior thalamic radiation and sagittal stratum, the latter associated with younger age of OCD onset, longer illness duration and higher percentage of medicated patients (Piras et al., 2019). Conversely, pediatric OCD was not linked to any microstructural abnormalities, which suggests that alterations in projection and association bundles to posterior brain, linked to medication status and disease chronicity, may be a consequence, rather than a cause, of OCD (Piras et al., 2019). Finally, a multimodal meta-analysis combining WM volume and FA reported widespread WM abnormalities, particularly evident in the anterior midline tracts, linking the anterior part of the cingulum bundle with the body of the corpus callosum (Radua et al., 2014).

Taken together, most of the above-mentioned studies accounted for the effect of medication status, comorbidities and age of OCD onset. However, the lack of harmonization of imaging and clinical data acquisition, together with limited details on the clinical characteristics of participants (e.g. treatment history, disease course, severity, symptom type, etc.), limits the ability to distinguish the effect of these specific

variables and, hence, may add variability to the imaging findings. Nevertheless, the development of meta-analyses and the large-scale data sharing facilitated by consortia like ENIGMA and OBIC have provided valuable knowledge on the neuroanatomical alterations of OCD.

## Structural MRI studies implementing the multidimensional model

To account for the clinical heterogeneity of the disorder some studies have opted for studying the structural correlates of the disorder implementing a multidimensional approach. Most studies assessing brain structural differences among OCD symptom dimensions have used a **VBM approach**. However, they provided highly heterogeneous findings and, unfortunately, to date, no single meta-analysis has combined these dimensional studies and provided more integrated results. Furthermore, a significant portion of VBM cross-sectional studies assessed dimensional differences by comparing patients with a predominant symptom dimension to healthy controls; however, they did not compare these patients to patients with other symptom dimensions. Hence, it is impossible to discern whether their findings reflect alterations specific of a symptom dimension or general of OCD. In this line, Szeszko et al. (2008) showed that patients with prominent *doubting/checking* and *contamination/cleaning* symptoms exhibited a similar pattern of structural alterations when compared to controls. Consequently, case/control studies, in this context, might reflect alterations characteristic of OCD samples, that persist in subgroups of patients with overrepresented symptom dimensions in general samples. For this reason, in this section we will only review correlational studies or studies comparing patients with different symptom dimensions, unless otherwise specified.

The first study using this approach reported that patients with predominant *doubting/checking* dimension showed smaller GM volumes in the right amygdala than patients with other symptom dimensions (Pujol et al., 2004). Subsequent correlational studies showed that this dimension negatively correlated with GM volumes in the right posterior cingulate cortex (PCC), the medial occipital cortex (Valente et al., 2005), the anterior temporal lobes (van den Heuvel et al., 2009; partially overlapping with the

findings from Pujol et al. (2004)), the bilateral insulae, the left inferior lateral OFC, the left putamen (Alvarenga et al., 2012) and the right cerebellum (Okada et al., 2015). Negative correlations were also observed between this dimension and WM volumes in the anterior temporal lobes and the bilateral PFC (van den Heuvel et al., 2009). Conversely, the *doubting/checking* dimension positively correlated with GM volumes in the left precuneus (van den Heuvel et al., 2009), the lateral parietal cortices (Alvarenga et al., 2012), and the right middle, inferior temporal and middle occipital gyri (Okada et al., 2015). However, none of these correlations were replicated in the largest VBM mega-analysis to date, which included 331 patients with OCD. In this study, *doubting/checking* symptoms positively correlated with GM volumes in the right lingual gyrus, and negatively with GM and WM volumes in the left superior parietal cortices (de Wit et al., 2014).

Regarding *contamination/cleaning* or *washing* symptoms, the results have been equally heterogeneous. Correlational studies have reported negative associations between this dimension and the GM volumes of the right premotor cortex (Gilbert et al., 2008), the bilateral dorsal caudate (van den Heuvel et al., 2009), the right insula (Okada et al., 2015), and the right thalamus (Hirose et al., 2017), and between this dimension and the WM volumes of the right parietal region (van den Heuvel et al., 2009). A single positive correlation was reported between *contamination/cleaning* and the GM volumes of the right cerebellar tonsil (Okada et al., 2015). Conversely, no significant correlations were observed for this dimension in other cross-sectional studies (Valente et al., 2005; Alvarenga et al., 2012), nor in the OBIC VBM mega-analysis (de Wit et al., 2014).

The *symmetry/ordering* dimension was negatively correlated with the GM volumes of the right posteroventral OFC, the right dorsomedial and pulvinar thalamic nuclei (Valente et al., 2005), the bilateral parietal, right motor and left insular cortices (van den Heuvel et al., 2009), and the left middle frontal and right lingual gyri (Okada et al., 2015). Conversely, this dimension positively correlated with GM volumes of the left lateral OFC and dorsal ACC (Valente et al., 2005), the right rectal gyrus (Okada et al., 2015) and to the GM and WM volumes of bilateral temporal regions (van den Heuvel et al., 2009). No correlations were found for this dimension in Gilbert et al. (2008) and Alvarenga et al. (2012). In contrast, the OBIC VBM mega-analysis reported only a negative correlation

between *symmetry/ordering* and the GM volumes of the left fusiform gyrus (de Wit et al., 2014).

The *sexual/religious* or *obsessing* dimension was negatively correlated with the GM volumes of bilateral ACC (Alvarenga et al., 2012), the right angular, the right inferior frontal and left parahippocampal gyri and the right cerebellum (Okada et al., 2015). In contrast, positive correlations were reported between this dimension and the right inferior and middle lateral OFC, the right dlPFC (Alvarenga et al., 2012), the right middle frontal gyrus (MFG) and the left precuneus (Okada et al., 2015). Again, none of these correlations were replicated in the OBIC VBM mega-analysis that reported a single positive correlation between this dimension and the GM volumes of the left middle temporal gyrus (de Wit et al., 2014).

Finally, the *hoarding* dimension was negatively correlated to the GM volumes of the left caudate body (Valente et al., 2005) and the right hippocampus (Alvarenga et al., 2012), and to WM volumes of the left angular gyrus (Hirose et al., 2017). Positive correlations were found between this dimension and the GM volumes of the left superior lateral OFC (Alvarenga et al., 2012) and the right precentral gyrus (Okada et al., 2015). As in the previous dimensions, these findings were not replicated in the OBIC VBM mega-analysis that reported a single negative correlation between *hoarding* and the GM volumes of the right cerebellum (de Wit et al., 2014).

To our knowledge, the only VBM study comparing GM and WM volumes of pediatric patients with *doubting/checking, contamination/washing* and *symmetry/ordering* found no significant differences across dimensions (Lázaro et al., 2014a).

It is important to highlight the high heterogeneity across VBM studies (displayed in **Table 1**). Such disparity can be partially explained by several variables, including methodological differences such as the significance level at which results were reported or the use of masks to restrict the analyses to certain brain areas. Moreover, the diversity of clinical tools employed to characterize symptom dimensions and severity, as well as the effect of chronic symptomatology, medication and comorbidities on brain structure may also have had an important influence on the diversity of findings across studies.

**Table 1.** Summary of previous voxel-based morphometry studies assessing Obsessive-Compulsive Disorder from a dimensional perspective

| Study | Sample | Analysis | Findings |
|---|---|---|---|
| Pujol et al., 2004 | 72 OCD | Comparison between patients with predominant aggressive/checking (n=30) (Y-BOCS checklist, in accordance to Mataix-Cols et al. factor analyses) and the rest of patients (p<0.05 FWE corr) | • Patients with predominant **aggressive/checking** symptoms had reduced GM volumes in the right amygdala compared to the rest of patients. |
| Valente et al., 2005 | 19 OCD | Correlation between symptom severity (Y-BOCS checklist, in accordance to Leckman et al. factor analyses) and GM volumes (p<0.001 uncorr) | • **Aggressive/checking** symptom severity negatively correlated with GM volumes of the right PCC and medial occipital cortex.<br>• **Symmetry/ordering** symptom severity positively correlated with GM volumes of the left lateral OFC and dorsal ACC, and negatively correlated with GM volumes of right posteroventral OFC and right dorsomedial and pulvinar thalamic nuclei.<br>• **Hoarding** symptom severity negatively correlated with the GM volume of the left caudate body. |
| Gilbert et al., 2008 | 25 OCD | Correlation between symptom severity (OCI-R) and GM volumes (post-hoc analyses in SPSS p<0.05) | • **Contamination/cleaning** symptom severity negatively correlated with the GM volume of the right premotor cortex. |
| van den Heuvel et al., 2009 | 55 OCD | Correlation between symptom severity (Padua-IR (n=50) and Y-BOCS checklist (n=47), in accordance to Mataix-Cols et al. factor analyses) and GM and WM volumes (p<0.05 FWE corr; ^p<0.001, Ke=25 voxels) | • **Aggressive/checking** symptom severity positively correlated with the GM volume of the left precuneus and negatively correlated with GM and WM volumes of the anterior temporal lobules, and the WM volume of the bilateral PFC.<br>• **Contamination/cleaning** symptom severity negatively correlated with the GM volume of the bilateral dorsal caudate and the WM volumes of the right parietal cortex.<br>• **Symmetry/ordering** symptom severity positively correlated with GM and WM volumes of the bilateral temporal regions and negatively correlated with GM volumes of the bilateral parietal, ^right motor and ^left insular cortices. |
| Alvarenga et al., 2012 | 38 OCD | Correlation between symptom severity (DY-BOCS) and GM volumes (p<0.05 FWE corr) | • **Aggressive/checking** symptom severity positively correlated with GM volumes of the lateral parietal cortices and negatively correlated with GM volumes of the bilateral insula, left inferior lateral OFC and left putamen.<br>• **Sexual/religious** symptom severity positively correlated with GM volumes of the right inferior and middle lateral OFC and right dlPFC. It also negatively correlated with the GM volume of the bilateral ACC.<br>• **Hoarding** symptom severity positively correlated with GM volumes of the left superior lateral OFC and negatively correlated with GM volumes in the right hippocampus. |

| | | | |
|---|---|---|---|
| de Wit et al., 2014 | 331 OCD | GM and WM comparison between patients with distinct predominant dimensions (Y-BOCS checklist, dichotomized) (p<0.001; Ke=100 voxels) | • Patients with predominant **aggressive/checking** symptoms exhibited greater GM volumes in the right lingual gyrus and decreased GM and WM volumes in the left superior parietal cortices.<br>• Patients with predominant **symmetry/ordering** symptoms exhibited reduced GM volume in the left fusiform gyrus.<br>• Patients with predominant **sexual/religious** obsessions exhibited greater GM volume in the left middle temporal gyrus.<br>• Patients with predominant **hoarding** symptoms exhibited decreased GM volumes in the right cerebellum. |
| Lázaro et al., 2014a | 62 OCD (children and adolescents) | GM and WM comparison between patients with distinct predominant dimensions (CY-BOCS in accordance with Leckman et al. factor analyses) (p<0.05 FWE corr) | • No significant differences in GM or WM volumes between patients with distinct predominant dimensions. |
| Okada et al., 2015 | 37 OCD | Correlation between symptom severity (DY- BOCS) and GM volumes (p<0.001 uncorr) | • **Aggressive/checking** symptom severity positively correlated with GM volumes of the right middle and inferior temporal and right middle occipital gyri and negatively correlated with the GM volume of the right cerebellum.<br>• **Contamination/cleaning** symptom severity positively correlated with GM volumes of the right cerebellar tonsil and negatively correlated with the GM volume of the right insula.<br>• **Symmetry/ordering** symptom severity positively correlated with GM volumes of the right rectal gyrus and negatively correlated with GM volumes of the left middle frontal and right lingual gyri.<br>• **Sexual/religious** symptom severity positively correlated with GM volumes of the right MFG and the left precuneus. It also negatively correlated with GM volumes of the right angular, right inferior frontal and left parahippocampal gyri and right cerebellum.<br>• **Hoarding** symptom severity positively correlated with the GM volumes of the right precentral gyrus. |
| Hirose et al., 2017 | 33 OCD | Correlations between symptom severity (OCI-R) and GM and WM volumes (q<0.05 FDR corr) | • **Contamination/cleaning** symptom severity negatively correlated with the GM volume of the right thalamus.<br>• **Hoarding** symptom severity negatively correlated with the WM volume of the left angular gyrus. |

ACC: Anterior Cingulate Cortex; CY-BOCS: Children's Yale-Brown Obsessive-Compulsive Scale; dlPFC: dorsolateral Prefrontal Cortex; DY-BOCS: Dimensional Yale-Brown Obsessive-Compulsive Scale; FDR: False Discovery Rate; GM: Grey Matter; Ke: Cluster extent; MFG: Middle Frontal Gyrus; OFC: Orbitofrontal Cortex; OCD: Obsessive-Compulsive Disorder; OCI-R: Obsessive-Compulsive Inventory Revised; Padua-IR: Padua Inventory Revised; PCC: Posterior Cingulate Cortex; PFC: Prefrontal Cortex; WM: White Matter; Y-BOCS: Yale-Brown Obsessive-Compulsive Scale.

Regarding **surface-based studies**, recent mega- and meta-analyses conducted by the OBIC and the ENIGMA-OCD working group have attempted to provide more robust dimensional correlates. The OBIC mega-analysis assessed adults with OCD and reported multiple specific cortical thickness increases in relation to distinct dimensions but could not find dimensional differences in subcortical volumes (Fouche et al., 2017). In contrast, subsequent ENIGMA studies assessed both pediatric and adult patients and reported an association between the *symmetry/ordering* dimension and higher surface area of the left cuneus in pediatric patients, but no dimensional differences in cortical thickness or subcortical volumes, although the latter was not assessed in pediatric OCD due to data limitations (Boedhoe et al., 2017; 2018). The lack of findings across studies regarding subcortical volumes might reflect that dimensional differences are more focal and, consequently, undetectable in surface-based analysis. However, only further research, especially in pediatric samples, will determine if this hypothesis is accurate.

Regarding cortical thickness, the OBIC mega-analysis (Fouche et al., 2017) found that the *doubting/checking* dimension correlated with increased cortical thickness in the right lateral occipital gyrus and with lower entorhinal thickness. The c*ontamination/cleaning* dimension was associated with increased cortical thickness in the left lateral OFC, precentral and right frontal gyri. The *hoarding* dimension was linked to increased cortical thickness in the bilateral IFG, left lateral OFC, superior parietal, middle temporal and lateral occipital gyri, right superior frontal gyrus and medial OFC and cuneus. The s*exual/religious* dimension was associated with increased thickness in the left isthmus of the cingulate gyrus, rostral middle frontal, lateral occipital, right lateral OFC, superior parietal and supramarginal gyri, and the precuneus. Finally, the s*ymmetry/ordering* dimension correlated with increased cortical thickness in the left insular, lingual, and pre- and postcentral gyri, as well as in the right medial OFC and the lateral occipital gyrus. It was also associated with decreased thickness in the right superior temporal gyrus. Associations between *doubting/checking* and *symmetry/ordering* dimensions with temporal and occipital regions concur with previous VBM findings (Valente et al., 2005; van den Heuvel et al., 2009), as well as positive associations between *sexual/religious* and *hoarding* dimensions with prefrontal and lateral OFC volumes (Alvarenga et al., 2012). However, most of these cortical thickness findings are not

consistent with VBM studies, possibly due to small sample sizes, clinical heterogeneity and methodological differences in the quantification of dimensions and imaging analyses. In contrast, the OBIC and ENIGMA mega-analyses shared a binary definition of the dimensions based on the Y-BOCS symptom checklist (i.e. present or absent). Nevertheless, the latter did not find symptom-specific differences in cortical thickness (Boedhoe et al., 2018). Such disparity of findings might be also related to methodological differences. More specifically, the ENIGMA mega-analysis used an atlas-based approach which segments whole structures using coarse parcellation, hence, it can be less sensitive to subtle regional alterations than the vertex-based approach implemented in the OBIC mega-analysis (Fouche et al., 2017; Boedhoe et al., 2018; van den Heuvel et al., 2020).

Among **DTI correlational studies**, the *doubting/checking* dimension was correlated to WM alterations around basal ganglia (Kim et al., 2015) and in the left IFG and middle temporal gyrus (Yagi et al., 2017), whereas the *hoarding* dimension was linked to decreased axial diffusivity (AD), which reflects axon integrity, in the right insular gyrus (Yagi et al., 2017). *Obsessing* symptoms correlated with lower FA in the corpus callosum and the cingulate bundle (Koch et al., 2012) and with decreased AD and MD in the right supramarginal gyrus (Yagi et al., 2017). Finally, *symmetry/ordering* was associated with lower FA integrity in the right inferior fronto-occipital-fasciculus (IFOF) and the right optic radiation (Koch et al., 2012). Moreover, this dimension positively correlated with the right precuneus FA but negatively with the radial diffusivity (RD) of this area, suggesting impaired myelination (Yagi et al., 2017). Regarding pediatric OCD, to our knowledge only a case-control study assessed dimensional differences using a DTI approach. They reported that patients with *doubting/checking* symptoms had decreased FA in the corpus callosum, the left ACC and the caudate nucleus, whereas patients with predominant *contamination/cleaning* exhibited decreased FA in the left midbrain, lentiform nucleus, insula and thalamus, and increased MD, AD and RD in the anterior lobes of the cerebellum and in the pons (Lázaro et al., 2014b). These studies suggest that some of the dimensional GM impairments reported in VBM or surface-based studies may extend to the surrounding WM.

Bringing all the structural studies together, the *doubting/checking* and *contamination/*cleaning dimensions have been consistently linked to GM and WM abnormalities in the basal ganglia. *Doubting/checking* has also been associated with GM and WM alterations in temporal regions. In agreement with this, checking behaviors have been hypothesized to stem from the inability to accurately recall whether an activity has been successfully completed (Rachman, 2002; van den Heuvel, 2009) and patients with such symptoms report memory impairments (Leopold and Backenstrass, 2015). Conversely, the *sexual/religious* or *obsessing* dimension has been linked with GM and WM impairments in cingulate and supramarginal areas, which are relevant for cognitive control. The s*ymmetry/ordering* dimension has been associated with GM and WM impairments in fronto-occipital areas, which may underlie the altered visual processing observed in patients with this dimension of symptoms. Interestingly, these associations are consistent with the neurocognitive differences among dimensions and support the notion that distinct clinical manifestations of OCD might be underpinned by partially different and specific brain regions.

## Resting-state functional connectivity studies assessing OCD as a homogenous clinical entity

Most of the studies assessing functional connectivity impairments in OCD have used a **seed-based approach**. This method involves extracting the BOLD time-series of previously selected ROIs to, subsequently, correlate them with the time-series of other brain areas (i.e. ROI-to-ROI approach) or with the rest of the voxels in the brain (i.e. seed-to-voxel approach). This allows to measure the degree of coherence or temporal correlation among them. For more information on the bases of functional MRI and resting-state functional connectivity, please see the Functional MRI section in the Methods of this thesis. In the case of OCD, most seed-based studies have selected seeds comprised within the CSTC circuit, although, to lesser extent, other networks of interest have been also examined.

The findings of multiple studies using striatal seeds have provided evidence of a CSTC circuit dysfunction in OCD patients and have shown distinctions in the cortical functional

connectivity pattern of ventral and dorsal striatal regions, consistent with the model of distinct sensorimotor, cognitive and limbic CSTC loops. Nevertheless, the directionality of such findings has been heterogeneous. For instance, some studies reported increased functional connectivity between the ventral striatum and the OFC of patients with OCD (Harrison et al., 2009; 2013; Sakai et al., 2011; Jung et al., 2013), which correlated with overall symptom severity (Harrison et al., 2009; 2013), whereas other studies found reduced connectivity between these areas that also correlated with symptom severity (Posner et al., 2014) and between the ventral striatum and the ventral tegmental area (Harrison et al., 2009).

Regarding the dorsal striatum, results have been equally heterogeneous, with one study reporting decreased connectivity between the dorsal caudate and the lateral PFC and between the dorsal putamen and the SMA (Harrison et al., 2009), and two studies, one of them assessing a pediatric sample of patients with OCD, reporting increased connectivity between the dorsal striatum and prefrontal, ventromedial frontal and parietal areas (Fitzgerald et al., 2011; Posner et al., 2014). Additional findings in youth with OCD include decreased connectivity between the dorsal striatum and the rostral ACC and between the dorsomedial thalamus and the dorsal ACC, specific to children with OCD aged between 8 and 12 (Fitzgerald et al., 2011). Reduced connectivity was also observed between the putamen and the OFC, the IFG, the insula and the operculum in adolescents (Bernstein et al., 2016), which concurs with findings in adults (Harrison et al., 2009). These findings are in agreement with the prevailing pathophysiological models of OCD and with the results reported in adult patients. Although further research is needed in the assessment of functional connectivity impairments in pediatric OCD, these findings highlight the importance of accounting for neurodevelopmental trajectories of CSTC circuits to accurately portrait the functional impairments in patients with OCD.

The medication status has been hypothesized to contribute to the heterogeneity of the above-mentioned findings. While Harrison et al. (2009; 2013) assessed medicated adult patients, Sakai et al. (2011) and Posner et al. (2014) enrolled patients that were under no pharmacological treatment at the time of the study. However, even in these latter studies, long-lasting medication effects could have acted as a confounder because in

Sakai et al. (2011) half of the patients were medication-naïve and the rest had been medication-free for 8 weeks, whereas in Posner et al. (2014) half of the participants were medication naïve but the rest had been off medication for 94 weeks. In the case of Fitzgerald et al. (2011), their findings persisted after excluding medicated patients from the analyses. Hence, the distinct patterns of altered striatal connectivity observed in OCD could be modulated by the illness course and the pharmacological treatment. Nevertheless, this is just a hypothetical interpretation of the discrepancies because studies assessing the effect of SSRIs on the functional connectivity between ventral striatum and vmPFC have shown no significant effects (McCabe and Mishor, 2011).

Seed-based studies assessing functional connectivity impairments of regions located in large-scale networks have mainly focused on the DMN. Consistently, they have reported decreased functional connectivity within regions comprised in this network. Importantly, this has also been observed in non-affected relatives of patients with OCD, which suggests that this feature could be an endophenotype of the disorder. Conversely, they found increased connectivity between areas of the DMN and the putamen, the insula and frontoparietal regions, which suggests that the DMN might interfere in the sensory, emotional and cognitive processing associated with these areas (Jang et al., 2010; Fitzgerald et al., 2010; Stern et al., 2012; Peng et al., 2014). More recently, a meta-analysis of seed-based resting-state functional MRI (fMRI), including 541 adult patients with OCD and 572 healthy controls, categorized each seed, based on its anatomical location, into the following predefined networks: DMN, FPN, SN, limbic network (LN), dorsal attention network, visual network and auditory-sensorimotor network, although the three latter had too few seeds for meta-analysis (Gürsel et al., 2018). They reported that adult patients with OCD exhibited hypoconnectivity within the FPN (peaking in the dlPFC) and the SN (peaking in the supramarginal gyrus) and between the FPN and the DMN (also peaking in the dlPFC) and between the SN, the FPN and the DMN (also peaking in the supramarginal gyrus). Furthermore, they reported within-network general dysconnectivity (i.e. no specific direction of connectivity change) in the FPN (peaking in the striatum) and the DMN (peaking in the vmPFC/ACC) and between-network general dysconnectivity between the FPN and the DMN (peaking in the vmPFC/ACC), the FPN and the LN (peaking in the OFC), the FPN and the SN (peaking in

the insula), and between the FPN and the dorsomedial thalamus, which was located outside the network mask. These findings were independent of age, age of OCD onset or medication. Importantly, between-network hypoconnectivity supports the three-network model of OCD pathophysiology, whereas the aberrant connectivity of prefrontal, striatal and thalamic regions corroborates the well-known CSTC model.

To a less extent, **Independent Component Analysis** (ICA) has also been used to assess functional connectivity impairments in OCD. In contrast to seed-based approaches, ICA is a data-driven method based on the decomposition of fMRI data into independent components, which allows to distinguish meaningful areas of correlated brain activity from physiological noise (Soriano-Mas and Harrison, 2017). Although ICA has the advantage of not relying on previously selected ROIs, which allows to explore more diffuse patterns of functional connectivity alteration across large-scale networks, the identification and separation of components (i.e. networks) can be challenging (Soriano-Mas and Harrison, 2017).

In the context of OCD, Cheng et al. (2013) reported that medication-naïve patients with OCD exhibited reduced functional connectivity within the DMN, mainly in the PCC and precuneus, and in the self-referential network, located mainly in the PFC. Conversely, they found increased connectivity in the self-referential network, located mainly in the thalamus/ACC. More recently, Fan et al. (2017) found that patients with OCD had increased connectivity between the SN and anterior parts of the DMN, between the SN and the dorsal FPN, as well as within several DMN, SN and FPN subsystems. Moreira et al. (2019) reported that patients with OCD exhibited decreased connectivity within visual and sensorimotor networks and between sensorial networks, but increased connectivity between DMN and cerebellar networks. Finally, Gürsel et al. (2020) found that, regardless of medication status, patients with OCD showed increased connectivity within the left FPN, mainly in the superior and MFG, and decreased connectivity between the left and right FPN. Conversely, when using a sliding-window approach they observed decreased connectivity between the left and right FPN, but also between the left FPN and the SN. These findings concur with the seed-based meta-analysis of Gürsel et al. (2018) in that they revealed connectivity impairments within and between large-scale brain networks (mainly in the DMN, FPN and SN). However, the directionality of

such impairments has been heterogeneous in ICA-based studies, possibly due to limited sample sizes (i.e. raging between 23 and 49 participants per group) and differing number of independent components.

Two groups have explored ICA-based functional connectivity alterations in youth with OCD. Weber, Soreni and Noseworthy (2014) found that medication-naïve youth with OCD exhibited increased connectivity within the auditory network and reduced connectivity in regions associated with the DMN compared to controls. Gruner et al. (2014) implemented a logistic regression to identify the components (i.e. networks) that maximally discriminated pediatric patients with OCD from age-matched controls. They identified three main networks: a middle frontal/dorsal anterior cingulate network, an anterior/posterior cingulate network, and a visual network, yielding an overall group classification accuracy of 76.1%. These findings were interpreted as indicative of the implication of the cingulate cortex and related control regions in the pathogenesis of OCD early in its course. Importantly, these results should be interpreted with caution due to the scarce number of studies using ICA-based approaches to assess youth with OCD, the methodological differences, and the very limited sample sizes (i.e. ranging between 9 and 23 participants per group).

Other approaches to assess functional connectivity alterations are **graph theory-based methods**. Graph theory is a field of mathematics focused on the modelling of complex networks. Broadly, complex networks can be represented as mathematical structures named graphs made up of vertices or nodes that are connected by edges or links. In the context of fMRI data, vertices correspond to voxels, clusters of voxels or anatomically defined ROIs, whereas edges correspond to the estimates of functional connectivity between them (Soriano-Mas and Harrison, 2017). Graphs can be characterized by their local and global properties. Local properties reflect the characteristics of individual vertices. Among them, the most used is the degree that represents the number of edges that are incident to a vertex, in the case of fMRI data, the number of voxels with which a particular voxel is significantly correlated. The degree is often used to determine the centrality of a vertex in the network. Hence, vertices with high degree (i.e. with a number of edges that exceeds the average), are considered hubs with a central role in the networks and disruptions in these vertices may alter the connectivity across the

network. In contrast, global properties reflect the overall organization of a graph. Clustering coefficient is a global metric designed to assess the division of a network into modules. Highly modular networks exhibit high connectivity between the vertices within modules but lower connectivity between vertices of different modules, consequently, local alterations in such networks would rarely translate into global disruptions. Path length is another global metric defined as the average number of steps along the shortest paths for all possible pairs of network vertices, which can be used as a proxy for integration within a network. Combining these metrics allows to characterize networks as reflecting "small-world" properties, which exhibit modularity (i.e. high clustering coefficient), but also efficient long-distance connections (i.e. short path length). This organization minimizes wiring costs, maximizes parallel processing and increases resilience to local disruptions (Soriano-Mas and Harrison, 2017).

The first study using this approach to assess functional connectivity alterations in OCD reported that the top-down control network of patients with OCD did not have an optimal small-world architecture. Specifically, they exhibited higher local clustering and functional connectivity increases in the control network and connectivity decreases in posterior temporal areas (Zhang et al. 2011). In agreement, Göttlich et al. (2014) reported that unmedicated patients with OCD displayed stronger connectivity within the executive/attention network and decreased connectivity within the LN and between the LN and the basal ganglia, the DMN, and the executive/attention networks, which might underlie the difficulty in reappraising intrusive thoughts. In contrast, Shin et al. (2014) reported reduced modularity within the FPN, however, these impairments normalized after treatment with SSRIs.

Other studies have found alterations congruent with the CSTC model of OCD however the directionality of their findings has been heterogeneous. For instance, Hou et al. (2014) and Tian et al. (2015) reported increased functional connectivity strength within the CSTC circuit, although Tian et al. (2015) also reported decreased connectivity in the left inferior OFC. Interestingly, Hou et al. (2014) found that patients with OCD and their first-degree relatives had an overlapping pattern of increased functional connectivity in the left OFC, bilateral caudate nucleus and left middle temporal gyrus, which suggests that these regions might be endophenotypes representing neuroimaging markers of

increased risk for OCD. In contrast, Jung et al. (2017) found decreased path lengths in the orbitofronto-striato-thalamic circuit, although posterior seed-based analysis showed increased functional connectivity between the medial thalamus and the striatum but decreased connectivity between the OFC and the dorsomedial striatum. Increased connectivity strength among subcortical areas of the CSTC was also reported by Beucke et al. (2013) and Anticevic et al (2014). Specifically, Beucke et al. (2013) segregated the functional connectivity in local and distant components and found that patients with OCD had increased distant functional connectivity strength in the subthalamic nuclei and increased local connectivity strength in the putamen. Interestingly, the latter was reduced after treatment. Similarly, Anticevic et al. (2014) reported increased connectivity in the right putamen and within the basal ganglia network, mainly in the dorsal striatum and anterior thalamus and, as in Beucke et al. (2013), it was reduced in medicated patients. It is worth mentioning that although Anticevic et al. (2014) found that connectivity strength was increased in the dorsal caudate, dorsal putamen and the thalamus, they also detected decreases in the ventral striatum, which supports the idea that CSTC loops might be differently affected in OCD.

Reports on the connectivity strength of the OFC in patients with OCD have been heterogeneous. For instance, Meunier et al. (2012) reported decreases within medial and lateral OFC and between this area and the dorsal ACC, premotor, sensorimotor and temporal cortices, whereas Beucke et al. (2013) found increases within medial and lateral OFC, both in local and distant connectivity strength, and between this area and the precentral and temporal cortices. In agreement with Meunier et al. (2012), Anticevic et al. (2014) reported reduced connectivity strength in the lateral PFC.

Findings in regions outside the CSTC circuit have been equally heterogenous, with increases and decreases in functional connectivity strength reported in the temporal and occipital cortices and the cerebellum (Hou et al. 2014; Tian et al., 2015). As in adults, youth with OCD also exhibited suboptimal small-world architecture, which suggests less efficient information transfer. Moreover, although modularity was lower in OCD, higher clustering was observed in frontopolar, supplementary motor and sensorimotor cortices and lower betweenness centrality at one frontopolar site (Armstrong et al., 2016). These findings are consistent with the hypothesis that OCD might be characterized by more

locally intensive connectivity in some regions coupled with less interaction with other brain regions. In general, results have been heterogeneous partially due to methodological differences and limited sample sizes. Hence, further research is needed, especially in pediatric OCD.

## Resting-state functional connectivity studies implementing the multidimensional model

To date, only two studies have examined dimensional differences among the functional connectivity patterns of patients with OCD (results displayed in **Table 2**). They both used **seed-based approaches** but selected different seeds of interest. The first study (Jang et al., 2010) included 22 unmedicated patients with OCD and assessed the functional connectivity of the DMN, using the PCC as the seed of interest. The authors found that *contamination/cleaning* symptoms positively correlated with the connectivity between the PCC and the right MFC and cuneus but negatively with the connectivity between the PCC and superior orbital gyrus. In contrast, *obsessing/checking* negatively correlated with the connectivity between the PCC and the bilateral MFG and right superior medial gyrus. Remarkably, the connectivity between the DMN and the right MFG correlated positively with *contamination/cleaning* and negatively with *obsessing/checking*. Additionally, the *obsessing/checking* dimension positively correlated with DMN connectivity in the left middle orbital gyrus, left superior frontal gyrus, right inferior temporal gyrus, and right calcarine gyrus. Finally, *symmetry/ordering* and *hoarding* dimensions positively correlated with the functional connectivity between the PCC and surrounding areas, including the cuneus and lingual and calcarine gyri. The second study (Harrison et al., 2013) involved 74 patients with OCD and assessed the functional connectivity of the ventral and the dorsal striatum. They found that *aggressive* symptoms, positively correlated with the functional connectivity between the ventral striatum and the vmPFC, and negatively with the functional connectivity between the ventral striatum and the bilateral anterior amygdala. S*exual/religious* obsessions positively correlated with the functional connectivity between the ventral striatum and the mid and anterobasal insular cortex. Finally, *hoarding* symptoms positively correlated

with the functional connectivity between the ventral striatum and the subgenual cingulate/medial OFC and between the dorsal striatum and distinct medial frontal regions.

A relevant difference between the two studies is the methodology used to characterize the symptom type and severity: Harrison et al. (2013) used the DY-BOCS to quantify symptom severity across 6 symptom dimensions, whereas Jang et al. (2010) used the 4 symptom dimensions model proposed by Bloch et al. (2008), based on a previous factorial analysis (Mataix-Cols et al., 1999), in which each dimension is scored as 0 (absent), 1 (present) or 2 (predominant). Considering the differences in symptom characterization and seed selection, it is impossible to integrate the findings reported in these studies. Nevertheless, they showed that symptom dimensions of OCD were associated with both common and distinct functional connectivity impairments. Future research using data-driven approaches and assessing the dimensional connectivity correlates of adults and youth with OCD, while also accounting for additional clinical variability (e.g. treatment history, duration of the disorder, comorbidities, etc.), will be relevant for advancing the current neurobiological models of OCD.

**Table 2.** Summary of previous resting-state seed-based functional connectivity studies assessing Obsessive-Compulsive Disorder from a dimensional perspective

| Study | Sample | Analysis | Findings |
|---|---|---|---|
| Jang et al., 2010 | 22 OCD | Correlations between symptom severity (Y-BOCS checklist, in accordance with Bloch et al. factor analysis) and functional connectivity from the PCC (within-group connectivity: q<0.05 FDR corr; post-hoc correlations: p<0.0125) | • **Obsessing/checking** symptom severity positively correlated with the connectivity between the PCC and the left middle orbital and superior frontal gyri, right inferior temporal and calcarine gyri. It negatively correlated with the connectivity between the PCC and the bilateral MFG and right superior medial gyrus.<br>• **Contamination/cleaning** symptom severity positively correlated with the connectivity between the PCC and the bilateral MFG and cuneus. It negatively correlated with the connectivity between the PCC and superior orbital gyrus.<br>• **Symmetry/ordering** and **Hoarding** symptom severity positively correlated with the connectivity between the PCC and the cuneus, lingual and calcarine gyri. |
| Harrison et al., 2013 | 74 OCD | Correlations between symptom severity (DY-BOCS) and functional connectivity from the ventral caudate, dorsal caudate, ventral putamen and dorsal putamen (p<0.05 FWE corr) | • **Aggressive** symptom severity positively correlated with the connectivity between the ventral striatum and the vmPFC, and negatively with the connectivity between the ventral striatum and the bilateral anterior amygdala.<br>• **Sexual/religious** symptom severity positively correlated with the connectivity between the ventral striatum and the mid and anterobasal insular cortex.<br>• **Hoarding** symptom severity positively correlated with the connectivity between the ventral striatum and the subgenual cingulate/medial OFC and between the dorsal striatum and distinct medial frontal regions. |

DY-BOCS: Dimensional Yale-Brown Obsessive-Compulsive Scale; Ke: Cluster extent; MFG: Middle Frontal Gyrus; OFC: Orbitofrontal Cortex; PFC: Prefrontal Cortex; PCC: Posterior Cingulate Cortex; vmPFC: Ventromedial Prefrontal Cortex; Y-BOCS: Yale-Brown Obsessive-Compulsive Scale

## 1.5 A developmental perspective of OCD: from subclinical to clinical expression of symptoms

It is important to acknowledge that the presence of obsessions and compulsions is not exclusive of individuals diagnosed with OCD. During normal development, children often engage in repetitive, ritualistic and compulsive-like behaviors that closely resemble obsessive-compulsive symptoms. This phenomenon is more frequent in 2 to 5-year old children when compared to their younger and older counterparts (Evans et al., 1997; Evans, Lewis and Iobst, 2004; Pietrefesa and Evans, 2007). Remarkably, distinct types of behavior exhibited different ages of onset. For instance, parents reported that around 22 to 25 months of age their children started engaging in arranging objects or performing certain actions until they felt "just right", which resembles the *symmetry/ordering* symptom dimension observed in OCD. Likewise, from 22 to 24 months of age, children began to be concerned with dirt or cleanliness, which reflects similar concerns to those expressed by patients with OCD and predominant *contamination/cleaning* symptoms. Conversely, around 25 and 27 months of age, children started to collect or store objects, which resembles the *hoarding* symptom dimension of OCD (Evans et al., 1997).

Although direct evidence linking the emergence of these behaviors at such early ages to later development of OCD is lacking, subsequent studies suggested that common neuropsychological mechanisms may underlie compulsive-like behaviors in normal development and in OCD. For example, similar to compulsive behavior in OCD, compulsive-like behaviors during childhood have been linked to fear and anxiety and seem to act as an emotion regulation strategy and a way to reduce anxiety (Evans et al., 1997; Evans, Lewis and Iobst, 2004; Pietrefesa and Evans, 2007). Moreover, OCD has been consistently associated with deficits in executive functioning, specifically in response inhibition, set-shifting and error monitoring. These functions experience a critical period of development between the ages of 3 and 5 years, which coincides with the peak occurrence of compulsive-like behavior in childhood. Similar to individuals with OCD, children exhibiting more compulsive-like behaviors perform worse on response

inhibition and set-shifting tasks compared to children with fewer ritualistic behaviors (Evans, Lewis and Iobst, 2004; Pietrefesa and Evans, 2007).

From a neurobiological perspective, response inhibition and set-shifting are supported by prefrontal, parietal and striatal regions (Norman et al., 2016; 2019), whereas performance monitoring is linked to the dlPFC and the ACC (Melcher, Falkai and Gruber, 2008). Alterations in these areas concur with prevailing neurobiological models of CSTC circuit impairment in OCD (Menzies et al., 2008; van den Heuvel et al., 2016). To date, no study has assessed the neural correlates of checking-like behaviors in children. However, a study hypothesized that they may stem from a critical interaction between inhibitory self-control development and an increased need to regulate anxiety (Evans, Lewis and Iobst, 2004). Specifically, the authors hypothesized that, as children mature, the lateral OFC/ACC functions that permit response inhibition and self-control may be also recruited to control negative emotions and reduce anxiety levels. Consequently, these demands may hijack lateral OFC function, reducing its flexibility and behavioral control capacity (Evans, Lewis and Iobst, 2004). The authors suggested that, after the age of 5, subsequent maturation of these areas may gradually allow more effective behavioral control, which would lead to anxiety reductions and significant decreases in compulsive-like behaviors (Evans, Lewis and Iobst, 2004). Nevertheless, evidence suggest that, although compulsive-like behaviors decrease after the age of 5, they do not completely disappear. In this line, a study reported a linear decrease in these behaviors from ages 3-4 to 11 years of age (Glenn, Cunningham and Nananidou, 2012).

Importantly, these compulsive-like behaviors have been considered adaptative in younger children as a mechanism to cope with anxiety (Evans, Lewis and Iobst, 2004) and even as a means to learn about the environment, contributing to the transition from pre-operational to concrete operational thinking in children younger than 7 years (Glenn and Cunningham, 2007). In contrast, these behaviors have been hypothesized to become problematic after this age, reflecting a continued reliance on previously effective coping mechanisms and a failure to transition to new and more adaptive strategies to relieve anxiety in the long-term (Evans, Lewis and Iobst, 2004). Nevertheless, the paucity of research assessing the neuropsychological and neurobiological signatures of compulsive-like behaviors in older children, together with

the use of different definitions and metrics to assess such behaviors, makes it impossible to determine whether these behaviors stop being adaptive and become indicative of pathology after the 7 years of age.

Interestingly, epidemiological studies have reported that subclinical obsessive-compulsive symptoms are present in 11-year-old children with an estimate prevalence of 8% and in adults with prevalence estimates ranging from 13% to 28% (Fullana et al., 2009; Ruscio et al., 2010). From a neurodevelopmental perspective, this could be explained by reports that found that response inhibition, as well as vlPFC activity elicited during this process, continue to mature from childhood through late adolescence (Nelson and Guyer, 2011). Moreover, the dorsal cognitive path of the CSTC circuit matures during late adolescence and early adulthood and, once matured, the processes relevant for cognitive control are assumed to shift from initial ventral regions to more dorsal areas (Arnsten and Rubia, 2012). Subclinical obsessive-compulsive symptoms could be limited to these vulnerable neurodevelopmental periods in which normative changes in areas impaired in OCD may elicit a similar symptomatology. However, this hypothesis does not explain the high prevalence of such symptoms in adults. Moreover, recent studies in adults with subclinical obsessive-compulsive symptoms reported that the severity of their symptoms positively correlated with the GM volume of the putamen (Kubota et al., 2016; 2019), which replicates a finding that has been consistently observed in adult individuals diagnosed with OCD (Radua and Mataix-Cols, 2009; Radua et al., 2010; Peng et al., 2012; Eng, Sim and Chen, 2015; Hu et al., 2017; Picó-Pérez et al., 2020). To date, it is impossible to ascertain the causes that lead to the development of subclinical-obsessive compulsive symptoms. Although in some cases they may resolve with age and the successful maturation of regions involved in the CSTC circuit, it is plausible that, in some cases, genetic predispositions and/or external environmental factors may lead to neurodevelopmental impairments in these areas resulting in long-lasting subthreshold symptoms.

Crucially, the relationship between subclinical obsessive-compulsive symptoms with OCD also remains unclear. Early reports suggested that subclinical obsessive-compulsive symptoms in children were not strong predictors of OCD (Valleni-Basile et al., 1996), yet, more recent studies with larger samples and longer follow-up times showed that

children reporting obsessions and/or compulsions at age 11 were more likely to meet diagnostic criteria for OCD in adulthood, 20 years later (Fullana et al., 2009), and that such likelihood increased with the number of symptoms (Ruscio et al., 2010). In this vein, retrospective studies have shown that individuals with OCD experience subthreshold obsessions and/or compulsions for an average of 5-7 years before meeting criteria for OCD (Coles, Johnson and Schubert, 2011; Thompson et al., 2020). Therefore, it is possible that **subclinical obsessive-compulsive symptoms may be a precursor to OCD in some individuals**, especially those at greater risk (e.g. offspring of individuals with OCD) (Black and Gaffney, 2008). This hypothesis is also supported by familial studies that found that these symptoms were more prevalent among first-degree relatives of individuals with OCD (Nestadt et al., 2000). Additionally, the specific obsessive-compulsive symptom profile observed in adults, in both subclinical and clinical contexts, have been developmentally linked to the same symptom precursors in childhood (Fullana et al., 2009), which supports the notion that **subclinical and clinical symptoms plausibly share common neural signatures, which may be explored from a dimensional perspective.** Considering the symptom similarities and, although less studied, the potential neuropsychological and neurobiological commonalities between subclinical and clinical obsessive-compulsive symptoms, subclinical obsessive-compulsive symptoms may be considered part of the OCD spectrum, possibly differing from OCD mainly in the degree of severity and impairment (Nestadt et al., 2000; Black and Gaffney, 2008).

Although the assessment of neural correlates of OCD has allowed to greatly advance in the understanding of this disorder, samples of patients with OCD usually present differences in key clinical characteristics, such as medication status, presence of comorbidities or course of the disorder, which have been suggested to be important confounders in these studies. In contrast, studying healthy children with subclinical obsessive-compulsive symptoms may have the advantage of allowing assessments free from the typical confounders observed in clinical samples. However, this approach poses its own difficulties, such as the interaction with rapid neurodevelopmental changes occurring in childhood and the associated between-sex dissimilarities. For instance, GM volume in frontal and temporal lobes peaks earlier in girls (i.e. at 9.2-9.5 years in frontal

lobes and at 9.9-10 years in temporal lobes) than in boys (i.e. at 10.1-10.5 years in frontal lobes and 11-11.6 years in temporal lobes). In turn, WM rapidly increases in both sexes until early childhood, becoming more gradual around 10 years of age (Lenroot et al., 2007; Tanaka et al., 2012). Between-sex maturation differences in GM development may have relevant implications for the manifestation of obsessive-compulsive symptoms. As stated in the beginning of this introduction, 10 years of age is the first peak for OCD diagnosis, however, boys constitute most of early-onset OCD cases, with nearly a quarter of them experiencing onset before 10 years of age. Conversely, girls have the highest slope of cases after 10 years of age, mainly during adolescence (Ruscio et al., 2010). A possible explanation may be that delayed GM development observed in boys could increase their vulnerability to early cognitive control impairment and, thus, to the development of obsessive-compulsive symptoms (Nelson and Guyer, 2011). Despite these difficulties, studying the neural correlates of obsessive-compulsive symptoms in healthy children could contribute to identify brain features that may eventually be associated with the development of OCD, and, therefore, serve to characterize populations of high-risk individuals. Nevertheless, only longitudinal studies of such samples will have the potential to identify brain features truly associated with the development of full-blown OCD.

## 1.6 Rationale for the study

As seen in the previous section, subclinical obsessive-compulsive symptoms are relatively common among the general population, both in youth and adults (Fullana et al., 2009; Ruscio et al., 2010), which contrasts with the much lower incidence of clinical OCD (Mathes, Morabito and Schmidt, 2019). Although subclinical obsessive-compulsive symptoms and clinical OCD largely differ in severity and impairment, they share similar phenomenology. Hence, subclinical obsessive-compulsive symptoms may be considered part of the OCD spectrum. Moreover, evidence indicates that subclinical obsessive-compulsive symptoms might be a precursor to OCD in some cases, especially in high-risk individuals (e.g. offspring of patients with OCD) (Black and Gaffney, 2008). Remarkably, specific symptoms dimensions observed in adults have been developmentally linked to

the same symptom precursors during childhood (Fullana et al., 2009; Ruscio et al., 2010), supporting the notion that subclinical and clinical obsessive-compulsive symptoms may have common neural signatures that can be explored from a dimensional approach.

Neuroimaging studies assessing the structural and functional correlates of distinct symptom dimensions in adults with OCD have provided relatively heterogeneous findings, probably due to multiple confounding factors. In children with OCD, such assessments have been scarce and performed in small samples. In contrast, the study of structural and functional correlates of distinct dimensions of subclinical obsessive-compulsive symptoms in large samples of children may allow to characterize the neural signatures of these symptoms without the interfering effects of medication, chronicity or comorbidities, while also accounting for the diversity of symptom profiles. Since structural and functional alterations associated with OCD seem to extend beyond specific brain areas, it is important to implement data-driven, whole-brain approaches in such an assessment. Moreover, it should also consider the rapid neurodevelopmental changes that occur during childhood and the associated between-sex differences at both anatomic and functional levels.

Furthermore, considering the genetic factors and the neurotransmitter impairment associated with OCD, the analysis of the structural and functional correlates of subclinical obsessive-compulsive symptoms can be further complemented with imaging transcriptomics techniques that will allow identifying the genetic signature of imaging changes and, consequently, provide a more integrated view of the different neurobiological underpinnings of subclinical obsessive-compulsive symptoms. Additionally, actual genomic data from the study samples can be combined with imaging transcriptomic findings to detect polymorphisms linked to symptom-related neuroimaging patterns potentially associated with an increased risk for OCD.

# Hypotheses and Aims

# 2. Hypotheses and Aims

## 2.1 Hypotheses

The paucity of previous research assessing obsessive-compulsive symptoms in healthy children has made our studies somewhat exploratory. Nonetheless, we defined specific hypotheses for each study on the bases of previous research implementing similar methodologies and assessing obsessions and compulsions in clinical samples.

**STUDY 1: Brain structural correlates of subclinical obsessive-compulsive symptoms in healthy children**

Hypotheses:

- Since previous attempts to link brain structural features with specific symptom dimensions in individuals with OCD have provided heterogeneous findings, we did not put forward a definite hypothesis about these results. However, given the notion that distinct symptoms may arise from impairments in different brain regions, we hypothesized that distinct symptom dimensions will be associated with specific structural changes in regional GM and WM volumes.
- Considering the rapid neurodevelopmental changes occurring during the ages of our sample (8-12 years) and the associated between-sex dissimilarities, we also hypothesized that age and sex will modulate the relations between symptoms and regional GM and WM volumes.

**STUDY 2: Brain functional connectivity correlates of subclinical obsessive-compulsive symptoms in healthy children**

Hypotheses:

- Considering that prevailing neurobiological models of OCD emphasizing the role of CSTC dysfunction are largely based on functional data, we hypothesized that

obsessive-compulsive symptoms will be associated with global functional connectivity changes in cortical, striatal and thalamic areas.

- Given that previous attempts to link specific functional connectivity features with specific symptom dimensions have landed heterogeneous findings, we hypothesized that different symptom dimensions will stem from specific changes involving distinct CSTC circuits.
- Considering the rapid neurodevelopmental changes occurring during the ages of our sample (8-12 years) and the associated between-sex dissimilarities, we hypothesized that age and sex will modulate the relations between symptoms and functional connectivity patterns.

**STUDY 3: Neurogenetics of attractor dynamic patterns associated with obsessive-compulsive symptoms in healthy children**

Hypotheses:

Considering not only the prevailing model of CSTC dysfunction in OCD, but also the large body of evidence reporting glutamatergic dysfunctions in OCD, and the relevance of glutamatergic excitatory synapses in increasing the stability of attractor networks, the following hypotheses were put forward:

- Obsessive-compulsive symptoms will be associated with attractor connectivity changes in cortical, striatal or thalamic areas. Specifically, different symptom dimensions will be associated with patterns of attractor connectivity involving distinct CSTC circuits.
- Genes expressed in symptom-related attractor regions will be involved in the Glutamate/GABA neurotransmitter release machinery.
- SNPs that modify the expression of genes related to the Glutamate/GABA neurotransmitter release machinery in symptom-related attractor regions will modulate the link between symptoms and attractor connectivity, thus acting as protective- or risk-factors for obsessive-compulsive symptoms.

## 2.2 Aims

The overall aim of the present thesis is to provide new insights into the neurobiological correlates of obsessive-compulsive symptoms in healthy children in hopes that this information may eventually be linked to the development of OCD and, hence, serve to characterize populations of high-risk individuals. To that aim, we used different neuroimaging modalities and techniques (structural MRI and static and dynamic functional connectivity) to assess potential associations between such symptoms and brain features. Moreover, in the last study, we combined neuroimaging and gene expression data to provide a more integrated view of the factors underpinning the onset of obsessive-compulsive symptoms. The specific aims of each study are detailed as follows.

**STUDY 1: Brain structural correlates of subclinical obsessive-compulsive symptoms in healthy children**

Aims:

- To identify the brain volumetric features associated with total and symptom-specific obsessive-compulsive severity scores in a large sample of healthy children.
- To evaluate the potential modulatory effects of age and sex on the relationship between brain anatomy and total and symptom-specific obsessive-compulsive scores.

**STUDY 2: Brain functional connectivity correlates of subclinical obsessive-compulsive symptoms in healthy children**

Aims:

- To identify global functional connectivity features associated with total and symptom-specific obsessive-compulsive severity in a large sample of healthy children.

- To identify the precise functional connectivity patterns from the data-driven identified regions and their associations with total and symptom-specific obsessive-compulsive severity scores.
- To evaluate the potential modulatory effects of age and sex on the relationship between functional connectivity features and total and symptom-specific obsessive-compulsive scores.

**STUDY 3: Neurogenetics of attractor dynamic patterns associated with obsessive-compulsive symptoms in healthy children**

Aims:

- To identify attractors (i.e. brain nodes with increased pulling properties that cause dynamic functional connectivity to repeatedly converge into them) associated with total and symptom-specific obsessive-compulsive severity scores in a large sample of healthy children.
- To explore the genetic signature of total and symptom-specific obsessive-compulsive severity-related attractors.
- To detect genetic risk factors that modulate the association between attractor connectivity and total and symptom-specific obsessive-compulsive severity scores.

# Methods

# 3. Methods

In this section, we detail the recruitment protocol of participants and the psychometric measures obtained. We also describe the different neuroimaging and neuroimaging-gene expression interaction techniques and analysis protocols used across the three studies of this thesis (they are summarized in **Figure 3**, at the end of this section).

## 3.1 Sample description and psychometric assessment

The participants of all the studies included in this thesis were healthy school children recruited within the framework of the 'Brain development and air pollution ultrafine particles in school children (BREATHE)' project (European Commission: FP7-ERC-2010-AdG, ID 268479). The BREATHE project assessed the impact of air pollution in cities on the cognitive development of children (Amoly et al., 2014; Sunyer et al., 2015; Forns et al., 2016; Pujol et al., 2016; Alemany et al., 2017), although these data were not used in the studies presented in this thesis.

The BREATHE project selected 39 schools in the city of Barcelona (Spain) and invited all families of children without special needs in the 2$^{nd}$, 3$^{rd}$ and 4$^{th}$ primary grades to participate in the study trough a letter or a school presentation. 2,897 school children enrolled in the study with similar participation rates across classes and school vulnerability indexes, but not between public and private schools (Forns et al., 2016). From this initial sample of 2,897 children, 810 families showed interest in participating in a neuroimaging sub-study. 491 of them were successfully contacted by phone, and, from this pool, 263 children completed the imaging protocol.

During the recruitment process, children with special needs, mental health, neurological or other major medical conditions, according to reports from the schools' psycho-pedagogic office, were not considered for inclusion. Conversely, non-severe chronic conditions, like allergies, were not considered an exclusion criterion. Children with any contraindication to MRI scanning were also excluded from the neuroimaging sub-study.

Considering the prevalence of subthreshold ADHD symptoms (Balázs and Keresztény, 2014) and the frequent comorbidity of these symptoms with subclinical Obsessive-Compulsive symptoms in children (Zijlmans et al., 2017), we initially deemed that excluding all participants with ADHD symptoms would limit the generalizability of our findings. Consequently, we asked school teachers not to exclude children with ADHD symptoms but instead to indicate, for each child, the presence of these symptoms with an 18-item list, described in the next section. In parallel, parents or tutors completed the Strengths and Difficulties Questionnaire (SDQ). Finally, all children were interviewed by a trained psychologist or psychiatrist of the research team who decided, considering the DSM-IV criteria and the information provided by school teachers and parents or tutors, which children fulfilled criteria for OCD, ADHD or other mental health diagnosis. Through this approach, clinicians considered not only the presence of symptoms, but also their degree of impairment in daily life and their incidence in multiple contexts. According to these clinical criteria, none of the children of our sample met criteria for ADHD or any other psychiatric diagnosis, albeit 25 of them had subthreshold ADHD symptoms and 6 of them were taking methylphenidate hydrochloride or atomoxetine hydrochloride.

All parents or tutors signed the informed consent form approved by the research ethical committee (number 2010/41221/I) of the IMIM-Parc de Salut Mar, Barcelona, and the FP7-ERC-2010-AdG ethics review committee.

From the initial sample of 263 healthy children, 1 participant was excluded due to incomplete psychometric data. Moreover, 7 subjects in Study 1 and 10 subjects in Studies 2 and 3 were excluded based on image quality criteria. Therefore, the main sample of the **Study 1** consisted of 255 healthy children. Importantly, sensitivity analyses allowed to confirm that all findings remained significant after excluding the 25 children with ADHD symptoms. In contrast, in our second study we observed that functional connectivity correlates partially differed before (N=252) and after (n=227) excluding children with ADHD symptoms, hence, in **Studies 2 and 3** our main sample consisted of 227 participants.

## Psychometric assessment

***Obsessive-Compulsive Inventory-Child Version (OCI-CV):*** subclinical Obsessive-Compulsive symptoms were evaluated using the OCI-CV, a self-reported 21-item questionnaire designed to assess obsessions and compulsions in both clinical and non-clinical pediatric populations. OCI-CV includes the strongest items of the 42-item adult version and the wording is adapted to be used in children between 7-17 years old. Each item is rated in a 3-point Likert-type scale (0=never to 2=always) and assesses the presence of 6 symptom dimensions: *doubting/checking*, *hoarding*, *neutralizing*, *ordering*, *obsessing*, and *washing*. *Hoarding*, *neutralizing*, *ordering*, and *washing* symptoms are measured by 3 items each (0 to 6 points), *obsessing* symptoms are assessed by 4 items (0 to 8 points) and *doubting/checking* symptoms by 5 items (0 to 10 points) (see **Table 3**, at the end of this section). Hence, OCI-CV allows to have a sub-score for each symptom dimension and a total score of global obsessive-compulsive symptoms, obtained by adding all dimensional sub-scores (0 to 42 points). OCI-CV has shown strong internal consistency (α=0.81) and test-retest reliability (0.77 for the total scale and raging between 0.68 to 0.89 for the subscales; completed 1.5 weeks apart). Moreover, correlations with prevailing measures of pediatric OCD (i.e. Children's Yale-Brown Obsessive-Compulsive Scale and the National Institute of Mental Health Global Obsessive-Compulsive Scale) are statistically significant and moderate. OCI-CV was designed and validated by Foa et al. (2010) and translated and validated in a Spanish population by Rosa-Alcázar et al. (2014).

***Strengths and Difficulties Questionnaire (SDQ):*** before the clinical interview, the presence of mental health problems was assessed by parents or tutors using the SDQ. This questionnaire consists of 25 items grouped in 5 categories: 4 of them focused on measuring behavioral difficulties (i.e. emotional symptoms, conduct problems, hyperactivity/inattention and peer relationship problems) and 1 category assessing prosocial behavior. Each category has 5 items that are rated on a 3-point Likert-type scale (0=not true to 2=certainly true), obtaining a score between 0 to 10 for each dimension. The total difficulty score is obtained by adding the 20 items for difficulties (excluding prosocial behavior) and can range from 0 to 40. The SDQ has shown strong internal consistency (mean α =0.73; ranging between α=0.57 and 0.82 for the subscales)

and test-retest reliability (mean=0.62; raging between 0.57 to 0.72 for the subscales, completed 4 to 6 months apart). SDQ was designed and validated by Goodman et al. (1997). The official Spanish version was translated by the *YouthinMind* team and can be accessed free of charge at sdqinfo.com. It was validated by Rodríguez-Hernández et al. (2012).

***ADHD Rating Scale-IV (ADHD-RS-IV):*** ADHD symptomatology was assessed by teachers filling out the school version of the ADHD-RS-IV. This questionnaire consists of 18 items separate into two categories: inattention (9 items) and hyperactivity/impulsivity (9 items). Each item is rated on a 4-point Likert-type scale (0=never or rarely to 3=very often). Hence, inattention and hyperactivity/impulsivity subscales can range from 0 to 27 points, whereas global ADHD symptoms can be scored between 0 and 54 points. The school version of the ADHD-RS-IV has shown strong internal consistency ($\alpha$=0.94 for the total score, $\alpha$=0.96 for inattention, and $\alpha$=0.88 for hyperactivity/impulsivity) and test-retest reliability (0.90 for the total score, 0.90 for inattention, and, 0.88 for hyperactivity-impulsivity, completed 4 weeks apart). ADHD-RS-IV was designed and validated by DuPaul et al. (1998) and it was translated and validated by Servera and Cardo (2007).

Importantly, OCI-CV, SDQ and ADHD-RS-IV were not used as diagnostic scales in the studies included in this thesis, but to characterize general or specific mental health features in participants. Conversely, the clinical interview was used to diagnose and exclude children with OCD, ADHD or other psychiatric diagnosis.

**Table 3.** Items of the Obsessive-Compulsive Inventory-Child Version and dimensional factor loadings from exploratory factor analysis performed by Foa et al. (2010)

| Factor (% of variance accounted) | Number of item and brief description |
|---|---|
| Doubting/Checking (27.61%) | 4. Checking things<br>5. Doubting if did things<br>13. Worry did not finish things<br>15. Checking doors, windows and drawers<br>20. Doubting if did something "right" |
| Obsessing (12.41%) | 1. Cannot stop bad thoughts<br>11. Upset by bad thoughts<br>14. Upset by intrusive bad thoughts<br>18. Saying things in response to bad thoughts |
| Hoarding (11.34%) | 3. Collect stuff that gets in the way<br>7. Collect things that don't need<br>16. Difficulty discarding |
| Washing (8.26%) | 2. Compulsive washing<br>10. Worry about cleanliness<br>21. Wash more than others |
| Ordering (6.86%) | 8. Upset if things not in order<br>17. Upset if people move things<br>19. Need things in a certain way |
| Neutralizing (6.16%) | 6. Counting<br>9. Repeating<br>12. Repeating numbers |

## 3.2 Neuroimaging techniques

The emergence of MRI in the late 1970s marked a leap forward in quality compared to previous neuroimaging techniques. The main advantages of MRI are that, unlike Computed Tomography, SPECT and PET, MRI is a minimally invasive technique that does not require the use of ionizing radiations or radioactive substances, and has a high spatial and temporal resolution. Consequently, over the last decades, the use of MRI as a tool to study the structure and function of the brain has become customary in both medical and research settings. In parallel, there has been a continuous development in hardware, imaging methods, image processing and software used to analyze MRI and fMRI data, providing more complex and effective approaches to examine the neural correlates of mental processes and mental health disorders.

### 3.2.1 Structural MRI

MRI uses the natural magnetic properties of hydrogen protons, present in the water molecules of the human body, to generate detailed images of any tissue. When the body is placed inside a strong magnetic field, such as an MRI scanner, the hydrogen protons' axes align in parallel (or antiparallel) with the magnetic field, reaching equilibrium. Clinical MRI scanners come in different field strengths, usually raging between 1.5 and 3 Teslas, albeit scanners up to 7 Teslas can be used for specific research purposes. When radio frequency pulses are added to the magnetic field, using a frequency band including the proton's frequency of precession around its axis, the equilibrium is disrupted and, during this excitation period, some protons move from the low energy (parallel) state to a high energy state . When the radiofrequency source is switched off, protons return to the resting state (relaxation period), emitting a radio wave signal that corresponds to the energy differences between the two states. This signal is captured by the radio frequency coils and plotted on a grey scale to build up sectional MR images, using magnetic field gradients to allow a precise mapping of the relaxation signal onto specific spatial coordinates. Importantly, due to the variable concentration of protons, different

brain tissues present distinct relaxation periods, which allows to differentiate the brain tissue types (i.e. GM, WM and CSF in MRI) (Berger, 2002).

Multiple types of analyses allow to assess the brain structure based on these images. In this work, T1-weighted images were used in **Study 1** to detect regional GM and WM changes associated with the presence of obsessive-compulsive symptoms by means of a VBM approach. Moreover, given that structural images have higher spatial resolution than functional images, they were also employed to optimize spatial transformations applied to functional data (i.e. normalization) in **Studies 2 and 3**, allowing a better mapping from subject to standardized space.

## Data acquisition

The structural images used in **Study 1** (and in the rest of studies for registration purposes) were acquired with a **1.5-Tesla Signa Excite system** (General Electric, Milwaukee, WI, USA) equipped with an eight-channel phased-array. Although 3-Tesla scanners are currently customary in pediatric hospitals, we did not use a 3T magnet following the recommendations of the FP7-ERC Ethics Review Committee that attempted to limit the exposure of healthy children to magnetic fields. High-resolution 3D anatomical images were obtained using an axial T1-weighted 3D fast spoiled gradient inversion recovery-prepared sequence. A total of 134 contiguous slices were acquired with inversion time 400 ms; repetition time 11.9 ms; echo time 4.2 ms; flip angle 150; field of view 30 cm; 256 × 256-pixel matrix; slice thickness 1.2 mm. All images were inspected by an expert neuroradiologist to detect clinically relevant and gross anatomical abnormalities.

## Data preprocessing

Before any statistical analysis, the raw structural images must be preprocessed with previously validated algorithms to improve image quality and to standardize its geometric and intensity patterns. In **Study 1**, raw structural images were transferred and preprocessed using **MATLAB** (MATLAB v.2015a; The Math Works Inc, Natick, MA) and

**Statistical Parametric Mapping** (SPM) (SPM8; The Wellcome Department of Imaging Neuroscience, London, UK). The preprocessing pipeline consisted of tissue segmentation, normalization to Montreal Neurological Institute (MNI) space, modulation, and Gaussian smoothing. The output of each step was visually inspected to guarantee accurate preprocessing. For additional information on the preprocessing of structural images, please see the article corresponding to **Study 1**.

## Statistical analyses

***Voxel-based morphometry (VBM):*** VBM is a computational approach to neuroanatomy that quantifies the density of GM at a voxel-wise whole-brain level. It allows to detect regional GM differences between groups or in association with a specific variable. The use of VBM has significantly increased over the last years mainly because, unlike the GM volume analysis of predefined ROIs, it allows for a comprehensive measurement of GM throughout the entire brain. Although it is mostly used to assess GM, VBM can also be implemented to measure other brain tissues, such as WM or, more rarely, CSF.

In VBM, preprocessed structural images are included in a statistical model to identify brain regions significantly associated with the goal of the study. In this model, selection of nuisance covariates is crucial, for instance, total GM volume or total intracranial volume should always be included to ensure that findings reflect regional volumetric changes. Moreover, age and sex should also be added to remove potential associations between these variables and volumetric changes (Barnes et al., 2010).

In the context of this thesis, in **Study 1**, regional GM and WM volumes were inspected in relation to total and symptom-specific (i.e. *doubting/checking, hoarding, obsessing* and *ordering*) OCI-CV scores. Importantly, *neutralizing* and *washing* scores were not modeled because of lack of variance (mean<1; median=0). However, they were included as nuisance covariates in dimensional models, together with total GM (WM) volume, age and sex, which were accounted for in all models. These analyses were performed on **MATLAB** (MATLAB v.2015a; The Math Works Inc, Natick, MA) and **SPM** (SPM8; The Wellcome Department of Imaging Neuroscience, London, UK).

### 3.2.2 Functional MRI

The discovery that MRI could be made sensitive not only to brain anatomy but also to brain activity occurred about 30 years ago (Ogawa, 1990; 1992). Since its inception in 1990, the popularity of fMRI has increased exponentially, mainly because of its noninvasiveness and relatively high spatial resolution, which poses significant advantages to other functional techniques such as PET or Electroencephalography (EEG).

fMRI measures the hemodynamic response of the brain in relation to neural activity. Neuronal firing triggers a series of physiological changes in the brain vascular system that can be detected by different fMRI techniques. The primary form of fMRI relies on the blood-oxygen-level dependent (BOLD) signal. Since neurons do not have an internal energy reservoir, their firing causes a demand of energy intake and, consequently, triggers the hemodynamic response. During this process, active neurons receive more oxygen from the blood than inactive neurons, which leads to an increase in their ratio of oxygenated hemoglobin (oxyhemoglobin) relative to deoxygenated hemoglobin (deoxyhemoglobin). The different magnetic properties of oxyhemoglobin and deoxyhemoglobin (because of the presence of iron atoms) allows fMRI imaging to create a map of brain regions with increased activation (Ogawa, 1990; Huettel, Song and McCarthy, 2008).

BOLD fMRI has been primarily used to assess regional activation changes during task performance. In the case of OCD, a myriad of tasks has been implemented to assess both symptoms (i.e. through symptom provocation paradigms) and neuropsychologic impairments (i.e. with tasks that require inhibitory control, cognitive flexibility, error monitoring, emotional processing, etc.). However, task-based fMRI ignores the intrinsic activity of the brain, present even in the absence of externally prompted tasks, which has been argued to better capture the essence of brain function (Raichle, 2010). In contrast, resting-state functional connectivity captures synchronous patterns in the spontaneous fluctuations of the BOLD signal during rest, which reflect the functional organization of large-scale brain systems (Raichle, 2010). This approach has revealed new insights of the functional organization of the brain, both in health and illness

conditions (van den Heuvel and Hulshoff Pol, 2010; Woodward and Cascio, 2015). Regarding psychiatric disorders, evidence has shown that focal brain dysfunctions are rare. Conversely, most disorders have been linked to abnormalities in large-scale networks of spatially distant and interconnected brain areas, suggesting that they are disorders of brain connectivity. Such considerations highlight the need to understand brain dysfunction in psychiatric illness from a network-based perspective (Fornito and Harrison, 2012).

In the context of this thesis, functional data were used in **Study 2** to assess the resting-state functional connectivity correlates of total and specific obsessive-compulsive symptoms. In **Study 3**, resting-state functional data were used to identify brain nodes with increased attractor properties (i.e. attractors) associated with total and specific obsessive-compulsive symptoms. The precise fMRI analyses implemented in these studies are detailed below.

## Data acquisition

Functional images used in **Studies 2 and 3** were acquired during a resting-state paradigm in which children were instructed to relax, stay awake and lie still without moving while keeping their eyes closed. As in the case of anatomical data, functional images were obtained with a **1.5 Tesla Signa Excite system** (General Electric, Milwaukee, WI, USA) equipped with an eight-channel phased-array head coil and single-shot echo planar imaging (EPI) software. The resting-state-fMRI sequences consisted of gradient recalled acquisition in the steady-state with repetition time 2000 ms; echo time 50 ms; pulse angle 90°; field of view 24 cm; 64 × 64-pixel matrix; slice thickness 4 mm (inter-slice gap 1.5 mm). Twenty-two interleaved slices were prescribed parallel to the anterior–posterior commissure line covering the entire brain. For each participant, we acquired 180 whole-brain EPI volumes, for a total duration of 6 minutes. Furthermore, the first four (additional) images were discarded to allow magnetization to reach equilibrium.

## Data preprocessing and denoising

Similar to structural MRI data, resting-state fMRI data must be preprocessed before any statistical analysis. In **Studies 2 and 3**, functional images were preprocessed with the default MNI preprocessing pipeline implemented in the **CONN toolbox** (Whitfield-Gabrieli and Nieto-Castanon, 2012; Functional Connectivity SPM Toolbox v17; nitrc.org/projects/conn). This pipeline included realignment and unwrapping, slice-timing correction and outlier detection. The segmentation of structural images was applied to functional data, which were then smoothed. In a subsequent denoising step, realignment parameters, motion outliers, BOLD noise from WM and CSF and a linear detrending term were regressed out from functional data. Finally, we applied a band-pass filtering of 0.008Hz-0.09Hz. For detailed information on the preprocessing and denoising of functional images, see the articles corresponding to **Studies 2 and 3**.

## Statistical analyses

Resting-state functional connectivity measures temporal correlations of spontaneous BOLD signal between spatially distributed brain regions in the absence of a stimulus. This approach assumes that areas with synchronous activity form functional networks (i.e. resting-state networks) that underpin specific brain processes (Woodward and Cascio, 2015). As noted in the introduction, there are two broad methods used to examine functional connectivity: seed-based and data-driven approaches. Seed-based analyses are based on the a priori selection of ROI (i.e. seed) from which BOLD time-series data are extracted. Connectivity is calculated as the correlation between the time-series of the seed and the time-series of other ROI(s) or all other voxels in the brain. Conversely, data-driven approaches do not involve the selection of predefined brain regions and allow to study functional connectivity at a whole-brain, voxel-to-voxel level.

Moreover, thanks to recent advancements, the study of intrinsic connectivity within the brain has evolved from primarily static approaches to include dynamic measurements. Static functional connectivity approaches use the entire BOLD time series to derive the average connectivity between regions, assuming that functional networks are static over time. However, this approach might be insufficient to tackle the complexities of

functional connectivity, as research has shown that brain connectivity fluctuates over time (Chang and Glover, 2010). Consequently, over the last decade, there has been a growing interest in quantifying dynamic brain connectivity. Among the different statistical methodologies developed, the sliding window technique has become the most popular approach to assess dynamic functional connectivity. This technique allows to segment the BOLD time series in partially overlapping time windows and to assess functional interconnections between different brain areas within each window. Hence, the sliding window approach allows to assess temporally varying patterns of communication between brain regions, providing a more accurate representation of brain function (White and Calhoun, 2019; Savva, Mitsis and Matsopoulos, 2019).

In the context of this thesis, we implemented static voxel-to-voxel and seed-based resting-state functional connectivity analyses in **Study 2**, and a graph-theory based approach to assess dynamic resting-state functional connectivity in **Study 3**. The different statistical methods are detailed in the articles corresponding to **Studies 2 and 3**, respectively, and are summarized as follows:

***Intrinsic Connectivity Contrast (ICC) analyses:*** In **Study 2**, we first used the data-driven ICC protocol, which characterizes the strength of the connectivity pattern between each voxel and the rest of the voxels in the brain (Martuzzi et al., 2011). Then, the intrinsic connectivity patterns were inspected in relation to the OCI-CV total and dimensional scores. These analyses were performed with the **CONN toolbox** (Whitfield-Gabrieli and Nieto-Castanon, 2012; Functional Connectivity SPM Toolbox v17; nitrc.org/projects/conn).

***Seed-based analyses:*** In **Study 2**, we also performed post-hoc seed-based analyses using as seeds the clusters of voxels significantly associated with OCI-CV scores in the ICC analyses. Thus, we were able to characterize the regions in which the correlation with our seeds of interest was associated with total and symptom-specfic OCI-CV scores. Again, these analyses were performed with the **CONN toolbox** (Whitfield-Gabrieli and Nieto-Castanon, 2012; Functional Connectivity SPM Toolbox v17; nitrc.org/projects/conn).

***Stepwise Functional Connectivity analyses:*** In **Study 3**, we implemented a dynamic graph theory-based approach known as Stepwise Functional Connectivity (SFC) to assess the presence of brain nodes with increased pulling properties (i.e. attractors) that could cause the dynamic functional connectivity to repeatedly converge into them, consequently locking the brain in over-stable cognitive states and hindering the transition to new ones (Diez and Sepulcre, 2018). Briefly, a sliding window approach was applied to the BOLD time series and an association connectivity matrix was built for each time window. Next, total connectivity was segregated through triangle motifs into local connectivity, which reflects connectivity between a node and the nodes of the same module, and distant connectivity, which refers to the connectivity between a node and nodes outside its module. Hence, when in this thesis we refer to local and distant connectivity, these terms derive from network-based topology and not from Euclidean distances within the human brain (Diez and Sepulcre, 2018). Subsequently, SFC analyses were performed simultaneously under the local and distant conditions for all voxels in each time window. In these analyses, the association matrices of each time window were used to calculate the connectivity steps from each node to the rest of the nodes in the graph topological space until they reach a stable state. In our framework, a step refers to the number of links (edges) that belong to a path connecting two nodes (Sepulcre et al., 2012; Diez and Sepulcre, 2018). The mean of all SFC maps, containing the connectivity convergences of each time window, was computed to obtain a single local and distant SFC map per subject. In these final maps, increased dynamic SFC values in a given voxel indicate higher connectivity streams repeatedly converging at that specific voxel, proxy of attractor (Diez and Sepulcre, 2018). These analyses were performed with **MATLAB scripts** (MATLAB v.2020b; The Math Works Inc, Natick, MA). The dynamic attractor patterns were then inspected in relation to the total and symptom-specific OCI-CV scores, using **SPM** (SPM12; The Wellcome Department of Imaging Neuroscience, London, UK). We also explored the genetic signature of obsessive-compulsive symptom-related attractors in order to detect potential risk and protective factors. This approach is detailed below in the Neuroimaging-gene expression interaction section.

In all second-level analyses of **Studies 2 and 3** we introduced sex and age as nuisance covariates. Likewise, since motion outlier volumes were regressed out during the denoising step, to compensate for the different across-subject data acquisition points, we also covaried for the degrees of freedom from first-level analyses. Moreover, similar to our structural analyses, in the symptom-specific second-level analyses, *washing* and *neutralizing* were added as nuisance variables due to lack of variance, whereas *doubting/checking, hoarding, obsessing* and *ordering* were included as predictor variables.

### 3.2.3 Multiple comparisons correction

Most of MRI and fMRI data analyses are performed using a **massive univariate approach**, where a separate hypothesis test is performed at every brain voxel. Considering that a brain volume consists of roughly 100,000 voxels, on the order of 100,000 tests are typically performed at a single time. In many fields, tests statistics with p-values below 0.05 are considered sufficient evidence to reject the null hypothesis. However, using a voxel-wise α of 0.05 implies that 5% of the voxels will show false positive results, which means that we could expect on the order of 5,000 false positive results (Lindquist and Mejia, 2015). Without an appropriate **correction of multiple comparisons**, the resulting GM/WM volume or connectivity maps will include an abnormally high number of regions, leading to false positive findings and erroneous conclusions (Lindquist and Mejia, 2015). Earlier MRI and fMRI studies tried to solve this issue by using uncorrected yet more restrictive p-values than classical statistical analyses (usually a p-value<0.001). Nevertheless, in recent years, a significant number of methodologies has been developed to correct for multiple comparisons, and they have become an essential tool in the neuroimaging field.

The most well-known correction methods for multiple comparison are **Family-Wise Error (FWE) and False Discovery Rate (FDR) corrections.** They are both based on quantifying the likelihood of obtaining a false positive result. FWE is defined as the probability of obtaining at least one false positive result according to the number of tests performed. Conversely, FDR is defined as the proportion of false positive results among

all significant tests. Based on these metrics, other correction methods have been developed. For instance, the **small volume correction** significantly reduces the number of comparisons by limiting the hypothesis testing to voxels within specific ROIs and, therefore, restricting the number of voxels and tests. Another widely used approach is **cluster-extent based thresholding**, which relies on considering the statistical significance of a group of contiguous voxels instead of the significance of each single voxel. Statistically significant clusters are based on the number of contiguous voxels whose statistical value exceeds a pre-determined threshold. The rationale behind this approach is that real results tend to be spatially contiguous, while type I errors (i.e. false positives) lean towards an evenly distribution. Hence, the presence of clustering can be used as a criterion to distinguish real results from noise (Ward, 2000; Lindquist and Mejia, 2015). Finally, another fast-growing approach to deal with multiple comparisons is **non-parametric statistic permutation**. Briefly, permutation tests approximate the distribution of the maximum statistic. They do so by repeatedly resampling the observed data under the null hypothesis. Interestingly, we can permute the observed data by relabeling each observation among the set of observed outcomes. The probability of an outcome as or more extreme than the one observed, the p-value, is the proportion of statistic values in the permutation distribution greater or equal to that observed. The actual labeling used in the experiment is one of the possible labels, so if the observed statistic is the largest of the permutation distribution, the p-value is 1/N, where N is the number of possible labels of the initial randomization scheme (Nichols and Holmes, 2001). This approach provides substantial improvements in power and validity and rely on minimal assumptions about the distribution of the data. Nevertheless, they tend to be computationally intensive.

In the context of this thesis, different correction methods have been applied to protect against false positive results. Structural results in **Study 1** were corrected with a cluster-extent based thresholding approach named **AlphaSim**, implemented in the SPM REST toolbox (Ward, 2000; restfmri.net) and, to correct for the non-isotropic smoothness of structural data, the cluster-threshold was adjusted using the **Hayasaka correction**, implemented in the SPM-VBM8 toolbox (dbm.neuro.uni-jena.de/vbm). Functional connectivity results in **Study 2** were corrected with a non-parametric permutation-

based method named **Threshold-Free Cluster Enhancement** (TFCE) (Smith and Nichols, 2009) implemented in the SPM-TFCE toolbox v171 (dbm.neuro.uni-jena.de/tfce). Finally, the dynamic attractor patterns of **Study 3** were corrected by applying a **cluster-level FWE** approach.

## 3.3 Assessment of neurodevelopmental factors

As stated in the introduction, assessing obsessive-compulsive symptoms in healthy youths poses specific difficulties such as the interaction with rapid neurodevelopmental changes occurring during this age window and the associated between-sex dissimilarities. Therefore, in **Studies 1 and 2** we evaluated the modulatory effect of sex and age on our findings. First, we divided the sample according to sex (i.e. girls and boys) and age (i.e. below and above the median age). Then, we assessed the correlations between brain anatomy (**Study 1**) or functional connectivity (**Study 2**) and OCI-CV scores within each sex and age subgroup (i.e. girls, boys, younger and older). Likewise, to explore potential interactions between sex and age, we also assessed these correlations across younger girls, older girls, younger boys and older boys. All correlations were statistically contrasted using Fisher r-to-z transformations. Moreover, interactions between age and OCI-CV were also assessed in linear regression analyses. These analyses were performed with the **Statistical Package for the Social Sciences** (SPSS) (SPSS 23; IBM Corp, Armonk, NY).

Although clinical interviews revealed that no children of our sample met diagnostic criteria for a mental health disorder, including OCD and ADHD, 25 children exhibited symptoms of subthreshold ADHD and 6 of them were taking methylphenidate hydrochloride or atomoxetine hydrochloride. As mentioned earlier, subthreshold ADHD symptoms are relatively prevalent in children and they tend to co-occur with subclinical obsessive-compulsive symptoms (Balázs and Keresztény, 2014; Zijlmans et al., 2017). Hence, in order to distinct the neurobiological correlates specific of subclinical obsessive-compulsive symptoms in children, in **Study 1** all analyses were replicated excluding the 25 children exhibiting subthreshold ADHD symptoms, whereas in **Studies 2 and 3**, we directly excluded these children from our main sample.

## 3.4 Neuroimaging-gene expression interaction

Over the last two decades, the field of **imaging genetics** has emerged as a powerful strategy to study the link between genetic variations and brain structural and functional features, which in turn influences behavioral phenotypes such as psychopathology. The progress of imaging genetics is linked to the development and increased availability of dense molecular genetic SNP sampling, imputation and genome-wide sequencing, together with high resolution imaging. Moreover, the creation of international consortia has facilitated the collection of large samples and data sharing between researchers from different backgrounds (Smit et al., 2012).

Broadly, imaging genetics has focused on correlating allelic variation at one or more genetic loci with variation in one or more imaging-derived phenotypes, initially through candidate gene studies and, more recently, at a genome-wide level. This approach is based on the premise that genetic variations associated with an imaging-derived phenotype modulate gene expression and protein production, altering the cellular function and, ultimately, the imaging-derived phenotype (Arnatkevičiūtė, Fulcher and Fornito, 2019; Fornito, Arnatkevičiūtė and Fulcher, 2019). Nevertheless, multiple factors hinder the interpretation of neuroimaging-genetic interactions within this approach. For instance, gene expression levels vary widely across brain areas (Hawrylycz, et al., 2015), and they are susceptible to epigenetic modifications (e.g. associated with age or socio-environmental influences) (Arnatkevičiūtė, Fulcher and Fornito, 2019; Fornito, Arnatkevičiūtė and Fulcher, 2019).

Fortunately, the recent development of **atlases of human gene expression** that span a large fraction of the genome in a large number of brain areas has overcome these limitations. These atlases have brought the unprecedented opportunity to link actual gene expression to the whole-brain organization, leading to the emergence of the imaging transcriptomics field. While traditional approaches focused on correlating structural DNA variants and imaging-derived phenotypic variation, **imaging transcriptomics** allow identifying a set of genes with spatial expression patterns that overlap with specific brain structural or functional features (Arnatkevičiūtė, Fulcher and Fornito, 2019; Fornito, Arnatkevičiūtė and Fulcher, 2019). Among the different

transcriptomic atlases developed, the **Allen Human Brain Atlas (AHBA)** has become the most popular because it provides whole-brain, high resolution genome-wide expression values from six human specimens, quantifying more than 20,000 genes in 3,702 spatially distinct brain samples. Importantly, gene expression data is mapped into a unified 3D anatomic framework based on MRI, which facilitates evaluating spatial correlations between gene expression and neuroimaging patterns and also allows to divide such assessment into cortical and subcortical gene expression and neuroimaging patterns (Hawrylycz, et al., 2012; **Allen Human Brain Atlas**: human.brain-map.org).

Assessing the functional properties of a gene set that spatially overlaps with an imaging-derived phenotype is crucial to verify that the genes are functionally relevant and to discover shared functions among these genes. Multiple databases have been developed to functionally characterize gene sets through functional enrichment analysis. Due to its comprehensiveness, **Gene Ontology (GO)** is one of the most widely used functional enrichment tools. Functional annotations in GO are divided in three sub-ontologies: *Biological Processes*, which are centered on biological programs accomplished by multiple molecular activities (e.g. DNA repair or signal transduction); *Cellular Components*, that includes the locations relative to cellular structures in which a gene product performs a function (e.g. mitochondrion or ribosome); and *Molecular Function*, which includes the elemental activities of a gene product at a molecular level (e.g. catalysis or transport) (Ashburner et al., 2000; The Gene Ontology Consortium, 2019; **Gene Ontology:** geneontology.org). Hence, researchers can perform statistical tests for one or more sub-ontologies depending on the gene functional domain they are interested in exploring. The output displays a table listing the functional annotations overrepresented among a gene set.

So far, we have seen that MRI/fMRI data can be used in transcriptomic imaging and functional enrichment analysis to characterize the genes associated with an imaging-derived phenotype of interest. Nevertheless, if researchers have also collected genetic data from the sample, they can combine the aforementioned approaches with the analysis of the genetic data to detect expression-modifying polymorphisms that modulate the association between gene expression and structural or functional brain features in the sample. Importantly, since transcriptomic imaging is a data-driven

approach and the AHBA contains more than 20,000 protein-coding genes that could be associated with a specific imaging-derived phenotype, it is crucial to collect the GWA data of the sample, which contains the genome-wide set of genetic variants of each individual. The first step would be to locate all the genetic polymorphisms of the genes that contribute to the overrepresented functional annotations obtained in the functional enrichment analysis. There are multiple databases to retrieve the SNPs of a specific gene. However, if we are only interested in the SNPs that modify gene expression in the brain regions included in the imaging-derived phenotype, it is important to use a database that allow to retrieve the SNPs filtering in a tissue-specific manner. For instance, the **Genotype-Tissue Expression (GTEx)** portal is comprehensive public resource to study tissue-specific genetic expression and regulation. It provides access to data including gene expression, QTLs and histology images (The GTEx Consortium, 2013; **GTEx Portal V8**: gtexportal.org). Therefore, the single tissue eQTLs viewer implemented in the GTEx project portal can be used to locate the SNPs of interest. Nevertheless, the study of SNPs poses some difficulties: First, they are highly abundant throughout the genome, which can create a multiple comparisons problem. Second, SNPs can be in strong **linkage disequilibrium (LD)**. LD occurs when alleles from nearby genetic variants tend to be inherited together in a non-random, linked fashion. Due to their physical proximity, genetic variants in LD are less likely to be separated during recombination, consequently, the alleles of the variants are inherited together more often than expected (Slatkin, 2008; Machiela and Chanock, 2015). When SNPs are in strong LD, assessing the alleles of a few SNPs within a specific haplotype sequence allows identifying the alleles of the rest of the SNPs. Therefore, analyzing all the SNPs of a haplotype would provide redundant information (Takeuchi et al., 2005). In these cases, LD filtering can be a useful tool to reduce the large number of linked SNPs to a few representative ones that suffice to define the haplotype, while also accounting for the multiple comparisons problem. The SNPs dimensionality reduction can be performed with online tools such as **SNPClip**, which filter SNPs based not only on LD, but also on missingness, allele frequency and genome map regions (Machiela and Chanock, 2015; **SNPClip**: ldlink.nci.nih.gov/?tab=snpclip). The doses of the selected SNPs can be extracted from the GWA data of the sample and different statistical models can be built to assess how these genetic variants influence the imaging-derived phenotype or the

relationship between the imaging-derived phenotype and a psychometric or behavioral variable.

In the context of this thesis, we used this neuroimaging-gene expression interaction pipeline in **Study 3**. First, to explore the genetic signature of obsessive-compulsive symptom-related attractors and, second, to identify genetic risk factors (i.e. SNPs) likely contributing to the emergence of attractors and, consequently, to obsessive-compulsive symptoms. Finally, we assessed these potential risk factors in the GWA data of our sample.

## Genetic data acquisition and preprocessing

Genetic data were obtained within the framework of the BREATHE project. Genotyping procedure is detailed in (Alemany et al., 2017). Briefly, DNA samples were collected from saliva with the Oragene DNA OG-500kit (DNA Genotek) and were quantified through Quant-iTTMPicoGreen® dsDNA Assay Kit (Life Technologies). Genome-wide genotyping was performed in 1778 children using the Human Core BeadChip WG-330-1101 (Illumina) at the Spanish National Genotyping Centre (CEGEN). During preprocessing, **PLINK V1.9** (Purcell et al., 2007; PLINK V1.9: cog-genomics.org/plink/1.9) was used to ensure genotyping quality. Genetic variants were filtered by Hardy-Weinberg equilibrium ($p<10^{-6}$), allele frequency excluding minor allele frequency (MAF<1%) and a SNP call rate with a minimum of 95%. Polymorphisms were imputed with **IMPUTE2** (Howie, Donnelly and Marchini, 2009; IMPUTE2: mathgen.stats.ox.ac.uk/impute), taking the **1000 Genomes project** phase I integrated variant set (1000 Genomes Project Consortium, et al. 2015; 1000 Genomes Project: 1000genomes.org) as a reference haplotype panel. From the final genotyped sample, 149 subjects also had good-quality fMRI data. Hence, the neuroimaging-gene expression interaction analyses of Study 3 were performed in this final sample of 149 healthy school children.

## Statistical analyses

***Spatial similarity analysis**:* First, we used the microarray gene expression data from the **AHBA** (Hawrylycz, et al., 2012; AHBA: human.brain-map.org). to assess the spatial similarity between protein-coding genetic profiles and the local and distant obsessive-compulsive symptom-related attractor maps, separately. The aim of these analyses was to find which genes had a brain expression that matched the brain regions identified as obsessive-compulsive symptom-related attractors. Importantly, depending on the location of such attractors we limited the gene expression assessment to either cortical or subcortical genes.

In brief, the cortical or subcortical transcriptional profiles from the **AHBA** (Hawrylycz, et al., 2012; AHBA: human.brain-map.org) and the local and distant obsessive-compulsive symptom-related attractor maps were anatomically transformed into a common space and, then, a correlation approach was used to assess the spatial similarity value of each cortical or subcortical gene's expression with the local and distant maps, separately. Finally, a statistical threshold of more than 2 standard deviations above the mean of the spatial similarity distribution was used to identify cortical or subcortical genes the expression of which was spatially similar to the local and distant maps.

***Gene Ontology (GO) term enrichment analysis:*** Second, within the **GO term enrichment analysis tool** (Ashburner et al., 2000; The Gene Ontology Consortium, 2019; GO: geneontology.org), we performed Protein Analysis Through Evolutionary Relationships (PANTHER) Overrepresentation analyses (Mi et al., 2019) by entering the list of genes whose expression pattern spatially correlated to the obsessive-compulsive symptom-related attractors. This method explores whether the genes of an entered set are more likely to be associated to a certain functional annotation than what would be expected in a random gene set. Importantly, in the PANTHER Overrepresentation analyses, we selected *Homo sapiens* as the reference list and we performed Fisher's Exact tests ($p<0.05$, FDR-corrected, Fold Enrichment>2) for the *GO Biological Processes* annotation dataset.

***Selection and extraction of Single-Nucleotide Polymorphisms (SNPs):*** Third, we used the single-tissue eQTLs viewer implemented in the **GTEx project portal** (The GTEx

Consortium, 2013; GTEx Portal V8: gtexportal.org) to locate all the SNPs of the genes involved in overrepresented functions that modified gene expression in brain regions where we had previously detected obsessive-compulsive symptom-related attractor behavior. To ensure equilibrium, we performed a **Linkage Disequilibrium Filtering** with the large list of SNPs retrieved trough **SNPClip** (Machiela and Chanock, 2015; SNPClip: ldlink.nci.nih.gov/?tab=snpclip), which significantly reduced the number of SNPs of interest. The doses of these final SNPs were extracted from the GWA data with **PLINK V1.9** (Purcell et al., 2007; PLINK V1.9: cog-genomics.org/plink/1.9). Finally, we evaluated whether genetic variants (i.e. having versus not having copies of minor alleles of each SNP) moderated the association between obsessive-compulsive symptoms and attractor connectivity, using **SPSS** (SPSS 23; IBM Corp, Armonk, NY).

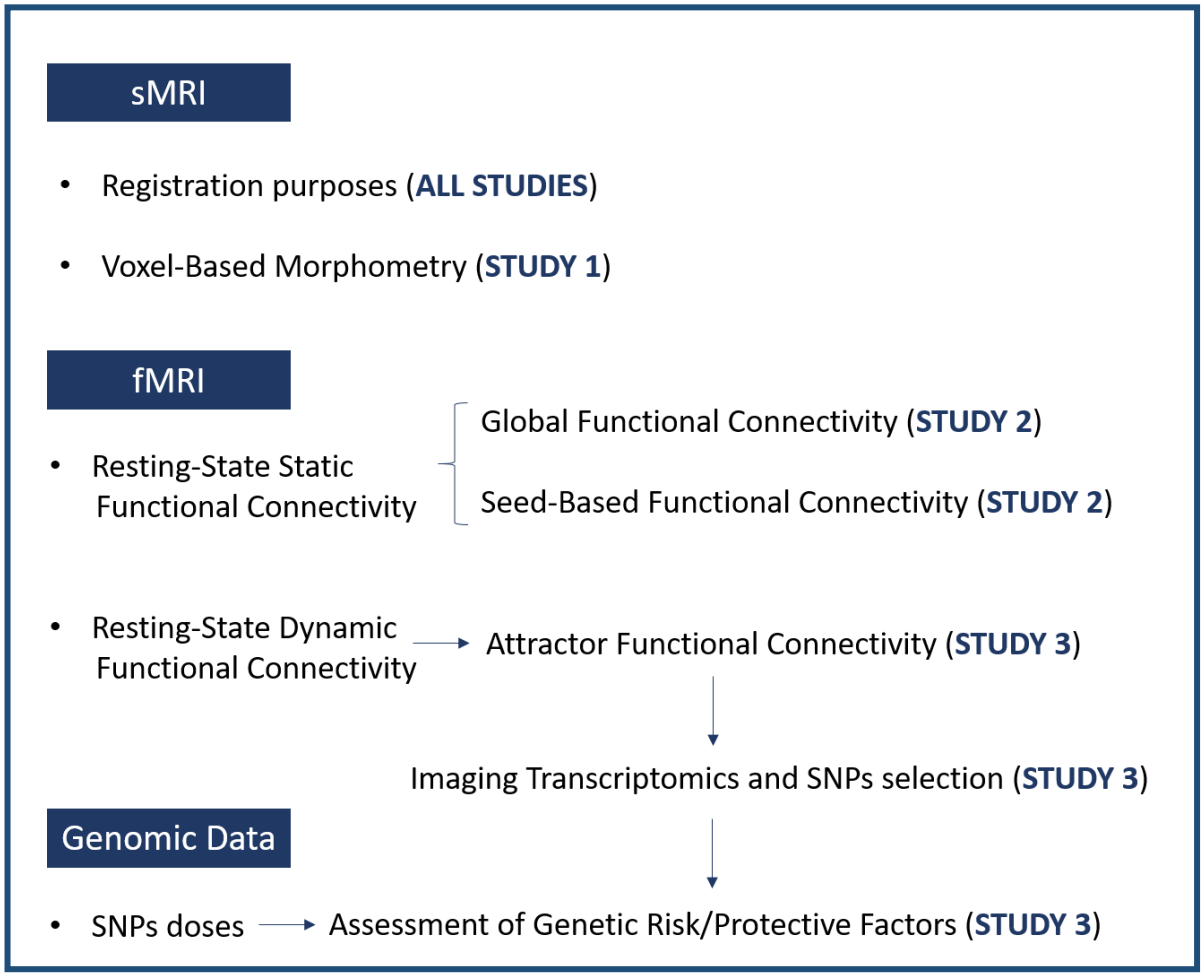


**Figure 3.** Scheme of the distinct neuroimaging and neuroimaging-gene expression interaction analysis techniques, specifying in which studies of this thesis they were implemented. fMRI: Functional Magnetic Resonance Imaging; sMRI: Structural Magnetic Resonance Imaging; SNPs: Single-Nucleotide Polymorphisms.

# Results

# 4. Results

## 4.1 Study 1

**Suñol M**, Contreras-Rodríguez O, Macià D, Martínez-Vilavella G, Martínez-Zalacaín I, Subirà M, Pujol J, Sunyer J, Soriano-Mas C (2018). Brain Structural Correlates of Subclinical Obsessive-Compulsive Symptoms in Healthy Children. J Am Acad Child Adolesc Psychiatry, 57(1):41-47. doi: 10.1016/j.jaac.2017.10.016.

NEW RESEARCH

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# Brain Structural Correlates of Subclinical Obsessive-Compulsive Symptoms in Healthy Children

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**Objective:** Subclinical obsessive-compulsive (OC) symptoms are frequently observed in children and have been reported to predict a subsequent diagnosis of OC disorder (OCD). Therefore, identifying the putative neurobiological signatures of such risk is crucial, because it would allow for the characterization of the underpinnings of OCD without the interfering effects of chronicity, medication, or comorbidities, especially when interpreted within the context of OCD clinical heterogeneity and taking into account normal neurodevelopmental changes. The present study aimed to identify the brain volumetric features associated with subclinical OC symptoms and the potential modulatory effects of sex and age in a large sample of healthy children.

**Method:** Two hundred fifty-five healthy children were assessed using the Obsessive-Compulsive Inventory–Child Version and underwent a brain structural magnetic resonance examination. The relation between total and symptom-specific scores and regional gray and white matter (GM and WM) volumes was evaluated. Participants were grouped according to sex and age (younger versus older) to assess the effect of these factors on symptom–brain morphometry associations.

**Results:** Ordering symptoms were negatively related to GM volumes in the ventral caudate. Hoarding symptoms were positively associated with GM and WM volumes in the left inferior frontal gyrus, and obsessing symptoms correlated negatively with GM and WM volumes in the right temporal pole. Doubt-checking symptoms correlated positively with WM volumes in the right inferior fronto-occipital fasciculus and the corpus callosum. Sex and age modulated some of these associations.

**Conclusion:** Subclinical OC symptoms are associated with specific brain volumetric features, which could be considered potential neural signatures of increased risk for OCD.

**Key words:** obsessive-compulsive disorder, symptom heterogeneity, subclinical symptoms, brain morphometry

**J Am Acad Child Adolesc Psychiatry** 2018;57(1):41–47.

Subclinical obsessive-compulsive (OC) symptoms are present in adults without a mental disorder diagnosis, with estimated prevalences ranging from 13% to higher than 28%.[1,2] These results are in clear contrast to the prevalence of OC disorder (OCD) in early adulthood (1.8%–2.3%).[1] Likewise, subclinical OC symptoms are present in 11-year-old children, with an estimated prevalence of 8%.[1] Despite early reports suggesting that subclinical OC symptoms were not strong predictors of OCD,[3] more recent studies with larger samples and longer follow-up times have found that children who reported obsessions or compulsions were significantly more likely to meet diagnostic criteria for OCD in adulthood,[1] and that such likelihood increases with the number of symptoms.[2] Therefore, it seems plausible that subclinical OC symptoms could be a precursor to OCD in some individuals who are at greater risk (e.g., offspring of individuals with OCD).[4]

Subclinical OC symptoms can be considered part of the OCD spectrum based on symptom similarity,[4] and familial studies have reported that subclinical symptoms are found at increased rates among first-degree relatives of individuals with OCD.[5] The difference with clinical OC symptoms probably lies in their severity and the lack of functional impairment associated with subclinical symptoms.[4,5] In this sense, OC symptoms observed in subclinical and clinical contexts in adulthood are reportedly developmentally linked to the same symptom precursors in childhood.[1] Therefore, subclinical and clinical symptoms conceivably share the same neural signatures, which can be explored within a dimensional framework.

The neural correlates of OCD in children have been evaluated in previous studies,[6] although such assessments have been typically performed in small samples and/or in the presence of multiple interfering factors (reviewed by Norman *et al.*[7]). Conversely, the assessment of the neural correlates of subclinical OC symptoms in children could allow for characterizing the underpinnings of OC symptoms without the interfering effects of chronicity, medication, or comorbidities. However, such an assessment would have to necessarily take into account the diversity of symptom profiles of this clinically heterogeneous disorder.[8] Most research on the clinical heterogeneity of OCD has approached the phenomenon from a dimensional perspective, which, compared with subtyping strategies, accounts for a larger percentage of the phenotypic variance of the disorder.[9] Moreover, dimensional approaches have important implications for the conceptualization of OC symptoms, because it is acknowledged that multiple symptom dimensions can coexist in a given individual. Likewise, it is assumed that a linear transition between the nonpathologic and pathologic expression of

symptoms exists, thereby upholding the assessment of subclinical OC symptoms in samples of healthy volunteers as nonpathologic manifestations of certain behavioral or cognitive features.[1] The development of assessment tools such as the Obsessive-Compulsive Inventory (OCI)[10] should be understood within this framework.

Structural magnetic resonance imaging has been widely used to assess the neural correlates of clinical heterogeneity in OCD. For example, checking symptoms have been associated with volume alterations in temporo-limbic regions, including the amygdala, insula, and putamen.[11-13] Similarly, washing symptoms have been associated with volume decreases in the dorsal caudate and the insula, and ordering symptoms have been associated with volume decreases in the sensorimotor cortex.[11,14] However, these results have been obtained in adult samples with an OCD diagnosis, and the study of the neural correlates of subclinical OC symptoms in children pose additional difficulties, such as the rapid changes in brain anatomy occurring at these ages owing to normal brain development and the possible between-sex differences in these processes.[2] For example, in frontal and temporal lobes, the peak of gray matter (GM) volume is reached at younger ages in girls than in boys (frontal lobes, 9.2∼9.5 years in girls versus 10.1∼10.5 years in boys; temporal lobes, 9.9∼10 years in girls versus 11∼11.6 years in boys).[15,16] Such between-sex maturation differences could have important implications for the manifestation of OCD symptoms. The delayed GM development observed in boys could increase their vulnerability to early cognitive control impairment and the development of OC symptoms.[17] Indeed, although 10 years of age is one of the peaks for OCD diagnosis, boys compose the majority of very early-onset OCD cases, with nearly one fourth of boys experiencing onset before 10 years of age (inflection point at 9.5 years[18]). In contrast, girls have a much more rapid accumulation of new OCD cases after 10 years of age (inflection point at 12.6 years[18]), with the highest slope during adolescence.[2]

To our knowledge, no studies have assessed the neural correlates of OC symptoms in young children, and only 1 previous study has performed a similar assessment in a sample of 1,938 healthy adolescents.[19] In that study, compulsive behaviors (including symptoms from different conditions, such as OCD or eating disorders) were associated with larger GM volumes in the bilateral orbitofrontal cortex and the right ventral striatum and dorsolateral prefrontal cortex. However, the investigators did not explore the relation between brain anatomy and different OC symptom dimensions or putative interactions with sex and age. In the present study, we recruited a large sample (N = 255) of healthy children with a mean age of 9.7 years and assessed the putative associations between brain anatomy and scores on different symptom dimensions (as assessed by the OCI–Child Version [OCI-CV]). Moreover, we explicitly evaluated age and sex interactions. Two general hypotheses were put forward: different symptom scores would be associated with specific structural changes in regional GM and white matter (WM) volumes, and age and sex would modulate the putative relations between OC subclinical traits and regional GM and WM volumes.

# METHOD

## Participants

The sample consisted of 255 healthy school children from the BREATHE project (European Commission: FP7-ERC-2010-AdG, ID 268479). The general project design is described elsewhere.[20] In brief, 1,564 families from 39 schools in the city of Barcelona, Spain were invited to participate in a study to assess the effects of environmental pollutants on normal neurodevelopment, although we did not use these data in the present study (see[21-25]). Children with special needs or mental health conditions, according to reports from the schools' psychologic pedagogic office, were not considered for inclusion. Importantly, to avoid bias selection, interested families (n = 491) were contacted from a random list with the sole limitation that each school was proportionally represented as a function of its number of children in the original sample. From this pool, 263 children completed the imaging protocol. Some individuals were excluded based on image quality criteria, leaving a final sample of 255 children (Table 1). Teachers of selected participants were asked about attention-deficit/hyperactivity disorder (ADHD) symptoms (*DSM-IV* scales; American Psychiatric Association, 2002), and parents were administered the Strengths and Difficulties Questionnaire[26] (SDQ; Table 1). In addition, all participants were interviewed by a mental health clinician to identify mental health alterations that could lead to a clinical diagnosis.

All parents or tutors signed the informed consent form approved by the research ethical committee (number 2010/41221/I) of the IMIM-Parc de Salut Mar, Barcelona, and the FP7-ERC-2010-AdG ethics review committee.

## Measures

Subclinical OC symptoms were assessed using the OCI-CV.[27] The OCI-CV is a self-reported 21-item questionnaire for the assessment of OC symptoms, which consists of 6 different subscales: doubt-checking, hoarding, neutralizing, obsessing, ordering, and washing (Supplement 1, available online).

## Imaging Data Acquisition and Preprocessing

See Supplement 2, available online.

## Data Analysis

Descriptive statistics of sociodemographic, psychometric, and global imaging variables (i.e., global GM and WM volumes) were analyzed using SPSS 20 (IBM Corp,

**TABLE 1** Sociodemographic Information, Brain Tissue Volumes, Strengths and Difficulties Questionnaire (SDQ), and Obsessive-Compulsive Inventory–Child Version (OCI-CV) Scores for the Study Sample

| | Mean ± SD | Median |
|---|---|---|
| Sample characteristics (N = 255) | | |
| Age (y) | 9.7 ± 0.88 (range 8–12.1) | 9.67 |
| Sex | | |
| Girls, n (%) | 125 (49) | |
| Boys, n (%) | 130 (51) | |
| School performance (0–5) | 3.74 ± 1.03 | 4.00 |
| Mother's education (0–5) | 4.52 ± 0.80 | 5.00 |
| Total GM volume ($cm^3$) | 712.75 ± 60.08 | 707.49 |
| Total WM volume ($cm^3$) | 460.27 ± 40.71 | 459.87 |
| SDQ scores (n = 253) | | |
| Emotional symptoms (0–10) | 1.88 ± 1.75 | 1 |
| Conduct problems (0–10) | 1.72 ± 1.64 | 1 |
| Hyperactivity/inattention (0–10) | 3.84 ± 2.50 | 4 |
| Peer relationship problems (0–10) | 1.18 ± 1.47 | 1 |
| Total difficulties score (0–40) | 8.63 ± 5.22 | 8 |
| Prosocial behavior (0–10) | 8.63 ± 1.51 | 9 |
| OCI-CV scores (n = 255) | | |
| Doubting-checking | 2.30 ± 1.53 | 2.00 |
| Hoarding | 2.05 ± 1.32 | 2.00 |
| Neutralizing | 0.39 ± 0.72 | 0.00 |
| Obsessing | 1.55 ± 1.40 | 1.00 |
| Ordering | 2.25 ± 1.59 | 2.00 |
| Washing | 0.64 ± 0.85 | 0.00 |
| Total | 9.17 ± 4.19 | 9.00 |

*Note*: *GM = gray matter; SD = standard deviation; WM = white matter.*

Armonk, NY). Between-group differences (i.e., between sex and age groups; see below) in these same variables were assessed with independent sample *t* tests and SPSS.

*Imaging: Global Sample Analyses.* We followed a voxel-based morphometry protocol to test the associations between total and symptom-specific OCD scores and regional GM and WM volumes. Specifically, we modeled 4 multiple regression models that allowed an assessment of the effects of the independent variables. Models 1 and 2 assessed the association between OCI-CV total score and voxel-wise GM (or WM) volume, with age, sex, and total GM (or WM) volume included as nuisance covariates.

The other 2 regression models included symptom-specific scores from the OCI-CV for cases in which a mean score higher than 1 was obtained: doubt-checking, hoarding, obsessing, and ordering. Neutralizing and washing scores were not modeled because of lack of variance (mean < 1; median = 0), although they were included as nuisance covariates. Age, sex, and total GM (model 3) or WM (model 4) volume also were included as nuisance covariates. Because the effects of predictor variables on voxel values were not estimated independently from each other, significance correction for the number of predictors was not deemed necessary.

*Imaging: Interactions With Sex and Age.* The study sample was divided in sex (125 girls and 130 boys) and age (below and above the median age of 9.67 years) subgroups, yielding 2 groups of 131 younger and 124 older children. Age effects also were explored using 2 additional cutoff points (10 years, peak of OCD diagnosis; 9.5 years, peak of OCD diagnosis in boys) to evaluate potential associations between regional brain volume and OC symptom effects specifically occurring before and after these clinically relevant developmental stages. With this approach, OCI-CV scores were modeled in interaction with sex or age, so the associations with regional GM and WM volumes were calculated within each subgroup and statistically contrasted using Fisher *r*-to-*z* transformation. Sex models included age and total GM or WM volume as nuisance covariates, and age models included sex and total GM or WM volume as confounders. To analyze the interaction between sex and age, correlations between brain anatomy and OCI-CV scores were assessed within 4 groups (i.e., younger boys [n = 66], older boys [n = 64], younger girls [n = 65], older girls [n = 60]).

Thresholding criteria for all these analyses are reported in Supplement 3, available online.

# RESULTS

## Descriptive Variables

Table 1 presents information on demographics, total GM and WM volumes, and SDQ and OCI-CV scores of participants. OCI-CV mean values of our sample were similar to those obtained in the Spanish validation of the questionnaire[28] (Table S1, available online). Clinical interviews detected no cases susceptible to receiving a diagnosis of a psychiatric disorder (including OCD), although 25 children (9.8%) had symptoms of ADHD (6 were taking methylphenidate hydrochloride or atomoxetine hydrochloride). SDQ scores from our sample did not differ from those of a normative population[29] (Table S2, available online).

## Brain Imaging Analyses

*Global Sample Analyses.* We observed no significant associations between overall OCI-CV scores and regional GM or WM volumes.

Subclinical ordering symptoms were negatively related to regional GM volumes in a cluster consisting of the ventral caudate nuclei bilaterally, including the nucleus accumbens and extending to the subgenual anterior cingulate cortex (Figure 1a). Hoarding OC symptoms were positively associated with regional GM and WM volumes in the left inferior frontal gyrus (Figures 1b and 2a), and obsessing symptoms correlated negatively with GM and WM volumes in the right temporal pole (Figures 1c and 2b). Doubt-checking OC symptoms correlated positively with WM volumes in 2 clusters located at the right inferior fronto-occipital fasciculus (IFOF) and the isthmus of the corpus callosum (Figure 2c, d). These same symptoms were negatively associated with GM volumes in a cluster involving the left middle frontal gyrus (Figure 1d), although only at a trending level ($p < .005$; Table 2).

Sensitivity analyses showed that these findings remained significant when excluding cases with ADHD symptoms (Table S3, available online).

*Interactions With Sex and Age.* Descriptive variables for age and sex groups are presented in Table S4, available online, and correlations between OCI-CV values and age are presented in Table S5, available

**FIGURE 1** Gray matter volumetric correlates of obsessive-compulsive symptoms. *Note:* Significant associations with ordering (a), hoarding (b), obsessing (c), and doubt-checking (d) symptoms are presented at a voxel-level significance of $p < .001$, except for ^$p < .005$. Color bars indicate *t* values. Hot colors correspond to positive associations, whereas cool colors correspond to negative correlations. Refer to online version of the article for full-color figure.

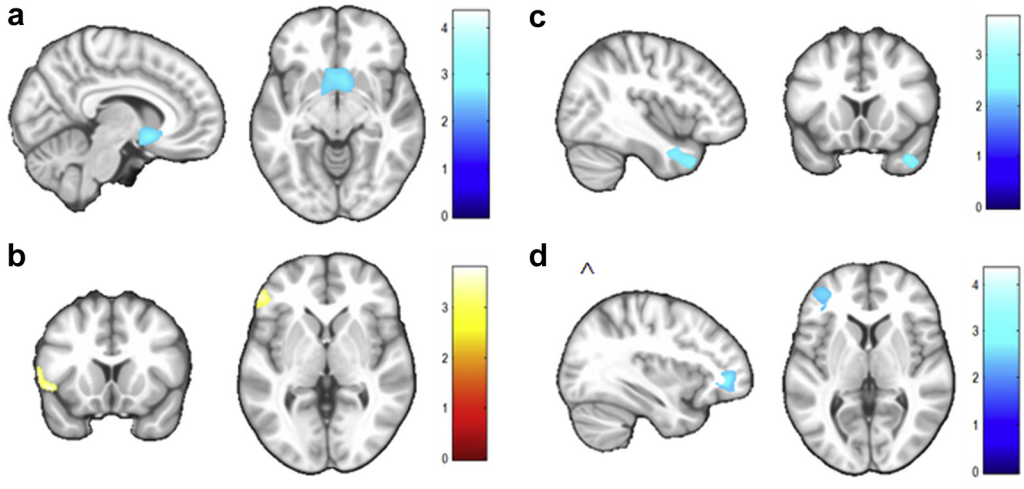
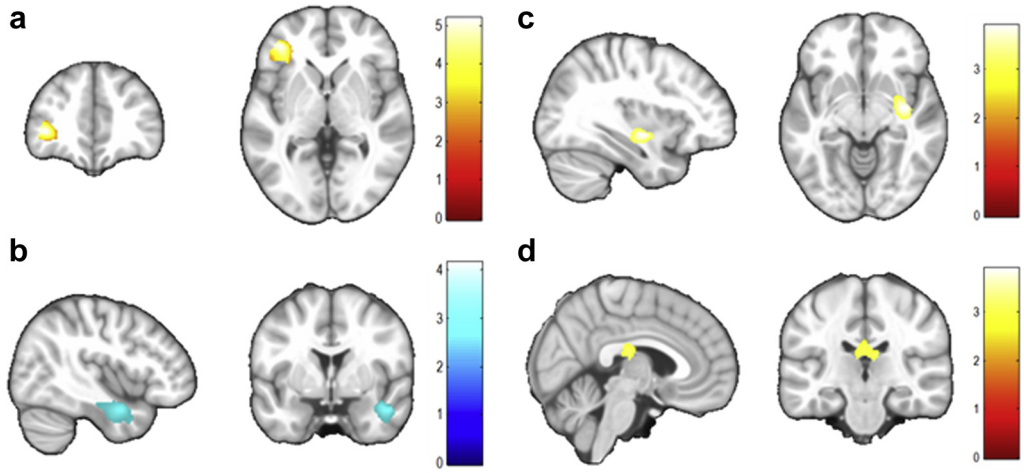


**FIGURE 2** White matter volumetric correlates of obsessive-compulsive symptoms. *Note:* Significant associations with hoarding (a), obsessing (b), and doubt-checking (c, d) symptoms are presented at a voxel-level significance of $p < .001$. Color bars indicate *t* values. Hot colors correspond to positive associations, while cool colors correspond to negative correlations. Refer to online version of the article for full-color figure.




online. A detailed description of whole-brain level interactions between OCI-CV scores/brain morphometric relations and sex and age is provided in Table S6, available online. Table S6 also presents those interactions overlapping with results from global sample analyses (indicated in italic type).

We did not observe significant interactions with sex for any of the clusters. Conversely, for interactions with age (using median age as a cutoff point), the positive correlation between hoarding symptoms and left inferior frontal GM volumes was specifically observed in older children (younger, $r = 0.01$; older, $r = 0.36$; $Z = 2.88$, $p = .004$). This result also was significant using the 10-year cutoff point, although in this case we also observed a positive association between hoarding symptoms and WM volumes in this same region in older children (Table S7, available online). The assessment of the interactions with sex and age showed that the negative association between ordering symptoms and GM volumes in the ventral caudate was specifically observed in the group of younger boys (younger boys, $r = -0.46$; other groups, $r = -0.14$; $Z = 2.44$, $p = .01$). This result also was significant using the 9.5-year cutoff point (Table S8, available online).

However, none of the interactions with age were found to be significant when considering age as a continuous variable (i.e., modeling symptom-by-age interaction in a multiple regression model). Specifically, we did not find significant linear or nonlinear (i.e., quadratic and cubic) interactions with age for any of the OC symptom dimensions.

## DISCUSSION

This is the first study to identify associations between subclinical OC symptoms and specific brain volumetric features in healthy children. We found associations between ordering, hoarding, obsessing, and doubt-checking symptoms and different GM and WM clusters. In addition, some GM findings were found to be moderated by the sex and/or age of the participants.

The negative association between ordering traits and GM volumes at the bilateral ventral caudate region resonates with previous clinical research demonstrating that deep-brain stimulation of this region ameliorates OCD symptoms.[30] Functional neuroimaging research also has shown that failure to activate the ventral caudate could account for the cognitive flexibility impairments observed in pediatric OCD samples.[31] Conversely, brain morphometry studies have reported that ventral striatum volume is increased in individuals with OCD[32] and showed a positive association with compulsivity scores.[19] However, such findings involve more lateral regions of the striatum (i.e., ventral putamen) and have been observed in adult OCD samples[32] or healthy adolescents.[19] Indeed, in clinical samples, ventral striatum volume increases interact with age, being most prominent in older adult samples.[13,33] Conversely, our findings specifically involved younger participants (<10 years of age). Similar to cortical regions, subcortical GM structures are larger (in relative terms) in pediatric than in adult samples, showing a volumetric decrease through adolescence and young adulthood.[34] Therefore, the negative association between ordering symptoms and ventral caudate volumes observed in the group of younger boys could be interpreted in neurodevelopmental terms as an alteration of the normal volume increase observed in subcortical structures throughout childhood. Moreover, the fact that this result was specific to boys could relate to the slower developmental rate of the ventral caudate in boys versus girls within this age range[35] and could elucidate the higher incidence of very early-onset OCD diagnoses (<10 years of age) in boys.[2] Notably, individuals with very early OCD onset are frequently characterized by the presence of ordering compulsions.[36] Therefore, the association between ventral caudate volumes and ordering symptoms reported in the present study could be considered the neurobiological correlate of the increased risk for developing OCD in boys younger than 10 years.

Hoarding symptoms showed a positive association with GM volumes in the left inferior frontal gyrus. GM content in this region has been found to be decreased in OCD[33]; therefore, the positive correlation observed with hoarding symptoms is somewhat unexpected. These contradictory findings could be explained in part by the limited presence of individuals with hoarding symptoms in studies with general OCD samples. In addition, although hoarding symptoms often begin during childhood and adolescence, there has been very little interest in investigating the neurobiological correlates of hoarding symptoms in youth[37]; hence,

**TABLE 2** Associations Between Specific Obsessive-Compulsive (OC) Dimension Scores and Regional Gray (GM) and White Matter (WM) Volumes

| OC Dimension | Brain Region | *x, y, z* | *t* | CS | Association/Tissue |
|---|---|---|---|---|---|
| Ordering | ventral caudate | 8, 11, −11 | 4.6 | 2,006 | −/GM |
| Hoarding | inferior frontal gyrus L | −53, 17, −5 | 3.8 | 885 | +/GM |
| | inferior frontal gyrus L | −39, 41, 2 | 5.2 | 1,219 | +/WM |
| Obsessing | temporal pole R | 39, 17, −35 | 3.75 | 574 | −/GM |
| | temporal pole R | 45, −2, −24 | 4.2 | 1,061 | −/WM |
| Doubt-checking | IFOF R | 36, −9, −8 | 3.9 | 695 | +/WM |
| | isthmus of corpus callosum | −3, −18, 17 | 3.4 | 558 | +/WM |
| | middle frontal gyrus L[a] | −35, 44, −3 | 4.3 | 3,834 | −/GM |

*Note: Anatomic coordinates (x, y, z) are given in Montreal Neurological Institute space. Positive and negative signs indicate positive and negative associations. CS = cluster size; IFOF = inferior fronto-occipital fasciculus; L = left; R = right.*

[a]*Voxel-level significance at* $p < .005$.

there is a lack of an appropriate context to interpret findings related to hoarding symptoms in samples such as ours. The inferior frontal gyrus is involved in response inhibition and emotional processing, and it is believed to regulate the activity of subcortical regions, thus affecting control over the selection and execution of actions.[38] In support of this idea, previous research has shown how this region shows aberrant activity in general OCD samples during tasks of cognitive control and conflict processing.[39] Although the exact link between these functions and hoarding symptoms is uncertain, our results provide preliminary evidence of the specific brain circuitry underpinning the development of hoarding symptoms. Moreover, the fact that we observed a positive association between GM volumes and hoarding symptoms specifically in the subgroup of older participants suggests that GM increases underlying these symptoms also could reflect neurodevelopmental disruptions, because with synaptic pruning, the GM content in the frontal lobes starts to decrease after 10 years of age as part of the normal developmental trajectory.[15] Importantly, however, as in the analyses of ordering symptoms, interactions with age were not observed in quantitative models and therefore should be interpreted with caution. We suggest that developmental effects do not progressively accumulate throughout the age range, but rather distinguish between developmental phases with different patterns of relations between brain structure and OC symptoms.

The presence of obsessing symptoms correlated negatively with the GM content in the right temporal pole. Interestingly, previous studies have reported the development of OC symptoms after temporal pole lesions.[40] More specifically, our finding is congruent with previous studies showing decreased volumes in the temporal pole associated with autogenous obsessions (phenomenologically similar to obsessing symptoms)[41] and with subclinical OC features in healthy controls.[42] The temporal pole has been related to complex cognitive processes such as moral cognition,[43] and activation of the temporal pole and other limbic/para-limbic regions seems to account for the exacerbated stress response observed in individuals with OCD with obsessing symptoms when facing moral dilemmas.[44]

We also should mention that, at a more lenient significance threshold, we observed that the increased presence of doubt-checking traits was associated with smaller GM volumes in a cluster located at the left middle frontal gyrus involving the ventrolateral prefrontal cortex. Previous studies in OCD samples have reported smaller volumes in this brain region,[45] and recent functional studies have shown that alterations of the ventrolateral prefrontal cortex could underpin deficits in cognitive flexibility (i.e., attentional set-shifting).[46] Interestingly, and in agreement with our findings, set-shifting abilities have been reported to be altered in those with OCD with checking symptoms in relation to other symptom subtypes,[47] and disruption of goal-directed (i.e., flexible) behavior has been specifically associated with subclinical checking symptoms in a sample of young adult healthy volunteers.[48]

Our analyses also provided a complex pattern of WM alterations associated with subclinical OC symptoms. Primarily, we observed a positive association between doubt-checking symptoms and WM volumes at the right IFOF and callosal isthmus. In agreement with our findings, the IFOF has been reported to show increased fractional anisotropy (a proxy of WM integrity) specifically in pediatric and adolescent OCD samples,[49,50] although opposite results have been observed in adult samples.[51] In this context, it is important to bear in mind that the developmental trajectory of WM differs from that of GM, and WM continues steadily increasing in volume up to adulthood.[52] Therefore, the pattern of results described earlier could be suggestive of an accelerated WM maturation in participants with OC symptoms, which probably could lead to enduring morphometric alterations in adulthood. The functional significance of such accelerated maturation remains elusive to our findings, although the IFOF is the major WM tract linking the ventrolateral and medial orbitofrontal cortices to the posterior parietal and occipital cortices; therefore, alterations in the IFOF could contribute to abnormal prefrontal functioning.[53] Importantly, this same pattern of increased versus decreased WM connectivity in child and adolescent versus adult OCD samples also has been observed in other brain regions,[54] which is suggestive of widespread WM maturation alterations in OCD. The corpus callosum is one of the regions where this abnormal maturation pattern has been reported (see, e.g., Fitzgerald *et al.*[55] for increased corpus callosum connectivity in pediatric OCD samples and Gan *et al.*[56] for opposite findings in adult samples). However, alterations in the callosal isthmus have not been typically observed in OCD, and our findings could be related to the delayed and protracted maturation of this region of the corpus callosum compared with more anterior regions.[57]

We also detected WM alterations congruent and overlapping with GM findings. Namely, WM content at the left inferior frontal gyrus was positively correlated with hoarding symptoms, and obsessing symptoms correlated negatively with WM at the right temporal pole. These findings indicate that the volumetric changes underpinning OC symptoms could extend to the afferent and efferent connections of these structures. Likewise, temporal pole findings should nuance the ideas in the previous paragraph, because they indicate that the relation between OC symptoms

and WM content in pediatric OCD samples is probably complex and region specific and do not always involve increases in WM connectivity.

This study is not without limitations. First, as in all correlational studies, we cannot infer causality from our findings, and therefore caution is warranted when interpreting the relations between brain anatomy and particular behavioral features. Moreover, some potential confounders might not have been entirely controlled for (e.g., subclinical alterations in affective state, familial history of brain abnormalities), and therefore their effect on our results cannot be ruled out. Second, the interpretation of our findings would probably benefit from the direct comparison with an age- and sex-matched group of participants with an OCD diagnosis. Such a comparison would allow us to ascertain whether the anatomic features described in association with OC symptoms also were present, and to what extent, in clinical populations. Likewise, a longitudinal assessment of the study participants would allow us to detect cases of individuals who develop OCD and therefore identify OCD biomarkers among the brain morphometry correlates of OC symptoms described in this study. Also, because we did not observe significant interactions with age in quantitative models, caution is needed to interpret the effects of developmental factors. Further research is warranted to elucidate whether age effects could be considered a significant modulator of the structural features associated with OC symptoms. Third, to further understand the neurobiological correlates of subclinical OC symptoms, other magnetic resonance imaging sequences (e.g., diffusion tensor imaging, functional imaging) should be used to obtain measurements that could complement the volumetric indices assessed in this study.

In summary, we have observed that different subclinical OC symptoms are associated with specific GM and WM features in children and adolescents. The present findings are in overall agreement with previous reports assessing clinical OCD populations, although previous reports on the neurobiological correlates of clinical heterogeneity in OCD have provided mixed findings. Nonetheless, the present results can be considered putative neural signatures of risk for developing OCD and could significantly contribute to the identification of at-risk individuals, which is crucial to developing targeted interventions to prevent the development or minimize the impact of OCD. Moreover, because the present findings have been obtained from a sample free of the potential confounding effects of medication, comorbidities, and chronicity, our results also could provide relevant information to refine and incorporate clinical heterogeneity into neurobiological models of OCD. Importantly, we also have observed that some of our findings interact with sex and normal neurodevelopmental processes; therefore, these factors must be taken into account when interpreting the results of studies assessing the neurobiological underpinnings of OC symptoms in populations of children and adolescents.

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**Supplementary Material**

**Supplement 1: The Obsessive-Compulsive Inventory-Child Version (OCI-CV)**

Responses in the OCI-CV are provided in a 3-point Likert-type scale (0=*never*—2=*always*) to increase sensitivity beyond simple yes/no responses. The OCI-CV has shown good internal consistency with $\alpha \geq 0.81$ for the total score and each of the subscales. Test-retest reliability coefficients are 0.77 for the total scale and range from 0.68 to 0.89 for the subscales. Correlations between OCI-CV and the gold-standard measures of pediatric obsessive-compulsive disorder (OCD), such as the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS) and the National Institute of Mental Health (NIMH) Global Obsessive-Compulsive Scale, are statistically significant and moderate.[1]

This questionnaire was considered optimal for the study purposes because it can be administered to healthy participants to assess subclinical symptoms, covers a wide age range (7 to 17), provides severity scores for six common OCD symptom dimensions, and is easy for children to understand and relatively quick to complete.

The OCI-CV assesses six different symptom domains:

**Doubting-Checking**: One of the most common dimensions of OCD. It is characterized by intolerance of uncertainty that leads to excessive worry and checking compulsions. The OCI-CV assesses this dimension with the following items: "I check many things over and over again," "After I have done things, I'm not sure if I really did them," "Even after I'm done I still worry that I didn't finish things," "I check doors, windows, and drawers over and over again," and "Even when I do something very carefully I don't think I did it right."
**Hoarding**: Characterized by obsessions and compulsions related to hoarding unnecessary objects. OCI-CV assesses this dimension with the following items: "I collect so much stuff that it gets in the way," "I collect things I don't really need," and "I don't throw things away because I'm afraid I might need them later."
**Neutralizing**: Involves counting and repeating compulsions generally associated with numbers. OCI-CV assesses this dimension with the following items: "I need to count while I do things," "I get behind in my schoolwork because I repeat things over and over again," and "I have to say some numbers over and over."
**Obsessing**: Involves intrusive thoughts that cause distress and fear. OCI-CV assesses this dimension with the following items: "I think about bad things and can't stop," "I'm upset by bad thoughts," "I get upset by

bad thoughts that pop into my head when I don't want them to," and "If a bad thought comes into my head, I need to say certain things over and over."

**Ordering**: Characterized by the presence of obsessions about symmetry or "just right" perceptions and ordering compulsions. OCI-CV assesses this dimension with the following items: "I get upset if my stuff is not in the right order," "I get upset if people change the way I arrange things," and "I need things to be in a certain way."

**Washing**: Characterized by the presence of contamination obsessions that are counteracted with compulsive and excessive cleaning. OCI-CV assesses this dimension with the following items: "I feel like I must wash and clean over and over again," "I worry a lot about things being clean," and "I wash my hands more than other kids."

## Supplement 2: Imaging Data Acquisition and Pre-Processing

Magnetic resonance imaging (MRI) acquisition was performed with a 1.5 Tesla Signa Excite system (General Electric, Milwaukee, WI, USA) equipped with an eight-channel phased-array head coil. Despite the fact that 3-Tesla exams are nowadays customary in pediatric hospitals, we did not use a 3T magnet, following the recommendations of the FP7-ERC Ethics Review Committee that attempt to limit the exposure of healthy children to magnetic fields. An axial T1-weighted 3D fast spoiled gradient inversion recovery-prepared sequence was obtained for each participant, with 134 contiguous slices and the following parameters: inversion time 400 ms; repetition time 11.9 ms; echo time 4.2 ms; flip angle 15º; field of view 30 cm; 256×256-pixel matrix; slice thickness 1.2 mm. Participants' heads were fixed inside the coil with foam pads to minimize motion artifacts.

Data were processed on a Microsoft Windows platform using technical software: MATLAB v.7.8 (The Math Works Inc, Natick, MA) and Statistical Parametric Mapping (SPM8; The Wellcome Department of Imaging Neuroscience, London, UK). Firstly, images were reviewed by an expert neuroradiologist to detect clinically-relevant and gross anatomical abnormalities. Moreover, in the course of image preprocessing (see below), a trained operator reviewed all the images to detect the presence of acquisition and pre-processing artifacts and magnetic field inhomogeneities (8 individuals were excluded because of poor quality exams).

Specifically, participants were excluded if the raw images showed obvious motion artifacts (ghost and blurring of the image), ringing or truncation artifacts, and susceptibility phenomena. After preprocessing, participants were excluded if the images showed brain anatomy deformations or truncated brain areas, non-optimal removal of non-brain tissue, or obvious tissue (gray/white matter [GM/WM]) misclassification. Next, images were preprocessed using a standard procedure including three main preprocessing steps: tissue segmentation, normalization to Montreal Neurological Institute (MNI) space, and Gaussian smoothing. Images were segmented using the "new segment" algorithm, and the rigidly transformed versions of GM and WM images derived from this algorithm were normalized using a Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra algorithm (DARTEL).[2] Specifically, using the option "create templates," images were iteratively matched to a template generated from their own average, so as to generate a series of templates with increasing resolution. Native space GM images from participants were then registered to the highest resolution GM template within a high-dimensional diffeomorphic framework. Subsequently, spatially normalized tissue maps were modulated by the Jacobian determinants from the corresponding flow-fields to restore the volumetric information lost during the high-dimensional spatial registration. Normalized images were transformed to the standard SPM template and re-sliced to a 1.5-mm resolution. Finally, images were smoothed with a 10-mm full-width at half-maximum isotropic Gaussian kernel.

**Supplement 3: Thresholding Criteria**

Spatial extent thresholds for all imaging analyses were determined by 1000 Monte Carlo simulations using the AlphaSim algorithm implemented in the SPM REST toolbox (http://restfmri.net). The input parameters were an individual voxel threshold probability of $p<.001$ (as recently suggested to prevent false positive findings),[3] a cluster connection radius of 3 mm (our neighborhood criteria for contiguous voxels included faces, edges, and vertices), and the actual data smoothness for each analysis. The output consists of a minimum cluster size (number of voxels) for each specific contrast. These cluster-extend thresholds are reported in the Results section. Moreover, considering that this is an exploratory study, in the global sample analyses we also explored volume correlates of obsessions and compulsions at a more lenient voxel height threshold of $p<.005$, although we acknowledge this threshold is below current recommendations.[4] In addition, in all cases, to correct for the non-isotropic smoothness of structural data, the spatial extent threshold was

adjusted using the Hayasaka correction[5] as implemented in the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm/).

**Table S1: Comparison of Obsessive Compulsive Inventory-Child Version (OCI-CV) Scores Between the Study Sample and the Spanish Validation Sample**[6]

| | **OCI-CV** | |
|---|---|---|
| **Our Project** | **N=255 (8-12y)** | |
| | *Mean ± SD* | *Median* |
| Doubting-Checking | 2.30 ± 1.53 | 2.00 |
| Hoarding | 2.05 ± 1.32 | 2.00 |
| Neutralizing | 0.39 ± 0.72 | 0.00 |
| Obsessing | 1.55 ± 1.40[a] | 1.00 |
| Ordering | 2.25 ± 1.59 | 2.00 |
| Washing | 0.64 ± 0.85 [a] | 0.00 |
| Total | 9.17 ± 4.19 [a] | 9.00 |
| **Spanish Validation Sample** | **N=914 (8-18y)** | |
| | *Mean ± SD* | *Median* |
| Doubting-Checking | 2.13 ± 1.64 | - |
| Hoarding | 1.90 ± 1.35 | - |
| Neutralizing | 0.32 ± 0.72 | - |
| Obsessing | 2.39 ± 2.01 | - |
| Ordering | 2.20 ± 1.68 | - |
| Washing | 3.10 ± 2.16 | - |
| Total | 11.34 ± 6.09 | - |

[a]Denotes a score significantly different from the score of the Spanish validation sample ($p<.05$, one-sample t-test).

**Table S2: Comparison of Strengths and Difficulties Questionnaire (SDQ) Scores Between the Study Sample and the Spanish Validation Sample**[7]

| **Our Project** | **Total N=253 (8-12y)** | | **Girls n=124** | | **Boys n=129** | |
|---|---|---|---|---|---|---|
| | *Mean ± SD* | *Median* | *Mean ± SD* | *Median* | *Mean ± SD* | *Median* |
| Emotional Symptoms (0-10) | 1.88 ± 1.75[a] | 1 | 1.93 ± 1.76 | 2 | 1.83 ± 1.75 | 1 |
| Conduct Problems (0-10) | 1.72 ± 1.64 | 1 | 1.43 ± 1.41 [a] | 1 | 2.00 ± 1.78 | 2 |
| Hyperactivity/Inattention (0-10) | 3.84 ± 2.50 [a] | 4 | 3.42 ± 2.26 [a] | 3 | 4.26 ± 2.66 | 4 |
| Peer Relationship Problems (0-10) | 1.18 ± 1.47 | 1 | 1.05 ± 1.34 [a] | 1 | 1.32 ± 1.58 | 1 |
| Total Difficulties Score (0-40) | 8.63 ± 5.22 [a] | 8 | 7.83 ± 4.86 [a] | 8 | 9.41 ± 5.45 | 8 |
| Prosocial Behavior (0-10) | 8.63 ± 1.51 | 9 | 8.86 ± 1.39 | 9 | 8.41 ± 1.59 | 9 |
| **Spanish Validation Sample** | **Total N=6,266 (34.10% aged 8-11y)** | | **48.59% Girls** | | **51.41% Boys** | |
| | *Mean ± SD* | *Median* | *Mean ± SD* | *Median* | *Mean ± SD* | *Median* |
| Emotional Symptoms (0-10) | 2.10 ± 0.06 | 2 | 2.19 ± 0.08 | 2 | 2 ± 0.08 | 2 |
| Conduct Problems (0-10) | 1.87 ± 0.05 | 1 | 1.83 ± 0.07 | 1 | 1.91 ± 0.07 | 1 |
| Hyperactivity/Inattention (0-10) | 4.19 ± 0.08 | 4 | 3.97 ± 0.12 | 4 | 4.39 ± 0.11 | 5 |
| Peer Relationship Problems (0-10) | 1.32 ± 0.05 | 1 | 1.33 ± 0.06 | 1 | 1.31 ± 0.06 | 1 |
| Total Difficulties Score (0-40) | 9.49 ± 0.18 | 9 | 9.34 ± 0.26 | 8 | 9.63 ± 0.25 | 9 |
| Prosocial Behavior (0-10) | 8.81 ± 0.04 | 10 | 9.04 ± 0.05 | 10 | 8.59 ± 0.07 | 9 |

[a] Denotes a score significantly different from the score of the Spanish validation sample ($p<.05$, one-sample t-test).

**Table S3: Results of the Sensitivity Analysis Excluding the 25 Children With Attention-Deficit/Hyperactivity Disorder Symptoms**

| Dimension | Brain region | x, y, z | t | CS | Association/ Tissue |
|---|---|---|---|---|---|
| Ordering | | | | | |
| | Ventral Caudate | 8, 11, -11 | 4.48 | 1529 | -/GM |
| Hoarding | | | | | |
| | Inferior Frontal Gyrus L | -48, 35, 2 | 3.85 | 387 | +/GM |
| | | -54, 18, -5 | 3.41 | 36 | +/GM |
| | Inferior Frontal Gyrus L | -41, 41, 2 | 5.32 | 1148 | +/WM |
| Obsessing | | | | | |
| | Temporal Pole R | 41, 6, -29 | 3.82 | 475 | -/GM |
| | Temporal Pole R | 42, 2, -21 | 4.17 | 746 | -/WM |
| Doubting-Checking | | | | | |
| | IFOF R | 36, -11, -8 | 3.18 | 9 | +/WM |
| | Isthmus of Corpus Callosum | -2, -18, 18 | 3.20 | 69 | +/WM |
| | Middle Frontal Gyrus L[a] | -36, 41, -3 | 3.77 | 717 | -/GM |

| Dimension | Group | Brain region | x, y, z | t | CS | Association/ Tissue | Fisher r-to-z | Correlation |
|---|---|---|---|---|---|---|---|---|
| **Age** | | | | | | | | |
| Hoarding | Older | Inferior Frontal Gyrus L | -57, 30, 5 | 4.39 | 532 | +/GM | Z=2.57 p=.01 | Y, r= -0.013 O, r= 0.319* |
| **Sex and age** | | | | | | | | |
| Ordering | Younger Boys | Ventral Caudate L | -9, 21, -8 | 3.39 | 42 | -/GM | Z=2.18 p=.03 | YB, r= -0.422* Other, r= -0.107 |

**Note:** GM = gray matter; IFOF = inferior fronto-occipital fasciculus; WM = white matter.

[a]Voxel-level significance at p<.005. *Denotes significant correlations (p<.05).

**Table S4: Demographic Information, Brain Volumes, Strengths and Difficulties Questionnaire (SDQ) and Obsessive Compulsive Inventory-Child Version (OCI-CV) Scores for Sex and Age Subgroups**

| Sample Characteristics | Girls (n= 125) | Boys (n=130) | Statistics | |
|---|---|---|---|---|
| | *Mean ± SD* | *Mean ± SD* | $t/x^2$ | *p* |
| Age (years) | 9.68 ± 0.88 | 9.72 ± 0.88 | 0.28 | 0.77 |
| School Performance (0-5) | 3.86 ± 1.01 | 3.62 ± 1.03 | 1.82 | 0.70 |
| Mother's Education (0-5) | 4.48 ± 0.89 | 4.56 ± 0.70 | 0.77 | 0.44 |
| Total GM volume ($mm^3$) | 680.49 ± 47.86 | 743.35 ± 54.30 | 9.79 | 0.00* |
| Total WM volume ($mm^3$) | 440.50 ± 33.65 | 479.07 ± 37.86 | 8.58 | 0.00* |
| **SDQ scores[a]** | | | | |
| Emotional Symptoms (0-10) | 1.93 ± 1.76 | 1.83 ± 1.75 | 0.44 | 0.66 |
| Conduct Problems (0-10) | 1.43 ± 1.41 | 2.00 ± 1.78 | 2.78 | 0.01* |
| Hyperactivity/Inattention (0-10) | 3.42 ± 2.26 | 4.26 ± 2.66 | 2.71 | 0.01* |
| Peer Relationship Problems (0-10) | 1.05 ± 1.34 | 1.32 ± 1.58 | 1.46 | 0.14 |
| Total Difficulties Score (0-40) | 7.83 ± 4.86 | 9.41 ± 5.45 | 2.43 | 0.02* |
| Prosocial Behavior (0-10) | 8.86 ± 1.39 | 8.41 ± 1.59 | 2.41 | 0.02* |
| **OCI-CV scores** | | | | |
| Doubting-Checking | 2.16 ±1.55 | 2.44 ± 1.51 | 1.46 | 0.15 |
| Hoarding | 2.10 ± 1.35 | 2.0 ± 1.29 | 0.58 | 0.56 |
| Neutralizing | 0.31 ± 0.69 | 0.46 ± 0.75 | 1.66 | 0.09 |
| Obsessing | 1.57 ± 1.38 | 1.52 ± 1.43 | 0.26 | 0.79 |
| Ordering | 2.18 ± 1.47 | 2.31 ± 1.70 | 0.62 | 0.53 |
| Washing | 0.65 ± 0.83 | 0.64 ± 0.84 | 0.09 | 0.93 |
| Total | 8.97 ± 4.02 | 9.37 ± 4.35 | 0.76 | 0.45 |

| Sample Characteristics | Younger (n= 131) | Older (n=124) | Statistics | |
|---|---|---|---|---|
| | *Mean ± SD* | *Mean ± SD* | $t/x^2$ | *p* |
| Age (years) | 8.99 ± 0.47 | 10.45 ± 0.50 | 24.06 | 0.00* |
| Sex | 65Girls (49.6%)<br>66 Boys (50.4%) | 60 Girls (48.4%)<br>64 Boys (51.6%) | 0.19 | 0.84 |
| School Performance (0-5) | 3.74 ± 1.00 | 3.73 ± 1.05 | 0.05 | 0.96 |
| Mother's Education (0-5) | 4.48 ± 0.87 | 4.56 ± 0.72 | 0.71 | 0.48 |
| Total GM volume ($mm^3$) | 711.61 ± 61.64 | 713.52 ± 58.57 | 0.25 | 0.80 |
| Total WM volume ($mm^3$) | 457.79 ± 41.53 | 462.67 ± 39.76 | 0.96 | 0.34 |
| **SDQ scores[a]** | | | | |
| Emotional Symptoms (0-10) | 1.88 ± 1.79 | 1.87 ± 1.72 | 0.07 | 0.95 |
| Conduct Problems (0-10) | 1.82 ± 1.67 | 1.61 ± 1.60 | 0.99 | 0.32 |
| Hyperactivity/Inattention (0-10) | 3.87 ± 2.44 | 3.84 ± 2.57 | 0.08 | 0.94 |
| Peer Relationship Problems (0-10) | 1.20 ± 1.46 | 1.17 ± 1.48 | 0.16 | 0.88 |
| Total Difficulties Score (0-40) | 8.77 ± 5.30 | 8.49 ± 5.15 | 0.42 | 0.68 |
| Prosocial Behavior (0-10) | 8.51 ± 1.60 | 8.76 ± 1.39 | 1.35 | 0.18 |
| **OCI-CV scores** | | | | |
| Doubting-Checking | 2.34 ± 1.58 | 2.27 ± 1.48 | 0.36 | 0.72 |
| Hoarding | 1.99 ± 1.29 | 2.10 ± 1.36 | 0.68 | 0.49 |
| Neutralizing | 0.46 ± 0.78 | 0.31 ± 0.66 | 1.59 | 0.11 |
| Obsessing | 1.66 ± 1.42 | 1.42 ± 1.37 | 1.39 | 0.16 |
| Ordering | 2.34 ± 1.60 | 2.15 ± 1.58 | 0.92 | 0.36 |
| Washing | 0.67 ± 0.87 | 0.61 ± 0.83 | 0.55 | 0.58 |
| Total | 9.46 ± 4.37 | 8.87 ± 3.98 | 1.12 | 0.26 |

**Note:** GM = gray matter; WM = white matter. [a]SDQ scores have two missing values; the subsamples in this particular case are 124 girls and 129 boys, and 130 younger and 123 older children. *Denotes significant between-group differences ($p<.05$).

**Table S5: Bivariate Correlations Between Obsessive Compulsive Inventory-Child Version Subscores and Age**

| | Doubting-Checking | Hoarding | Neutralizing | Obsessing | Ordering | Washing | Total |
|---|---|---|---|---|---|---|---|
| **Age** | r=0.026<br>p=0.682 | r=0.029<br>p=0.646 | r=-0.121<br>p=0.054 | r=-0.08<br>p=0.204 | r=-0.073<br>p=0.248 | r=-0.064<br>p=0.305 | r=-0.07<br>p=0.268 |

**Table S6: Whole-Brain Interactions of Obsessive-Compulsive (O-C) Symptoms–Brain Morphometry Associations With Sex and Age**

| Dimension | Group | Brain region | x, y, z | t | CS | Association/ Tissue | Fisher r-to-z | Correlation |
|---|---|---|---|---|---|---|---|---|
| **Age** | | | | | | | | |
| *Hoarding* | *Older* | *Inferior Frontal Gyrus L* | *-57, 30, 6* | *4.37* | *1765* | *+/GM* | *Z=2.88*<br>*p=0.004* | *Y, r= 0.009*<br>*O, r= 0.358** |
| **Sex and age** | | | | | | | | |
| *Ordering* | *Younger Boys* | *Ventral Caudate L* | *-9, 18, -9* | *4.22* | *1222* | *-/GM* | *Z=2.44*<br>*p=0.01* | *YB, r= -0.457**<br>*Other, r= -0.137* |
| Hoarding | Younger Boys | Genu of Corpus Callosum | 2, 17, 11 | 4.06 | 1374 | +/WM | Z=2.78<br>p=0.005 | YB, r= 0.362*<br>Other, r= -0.026 |
| Doubting-Checking | Older Girls | Superior Frontal Gyrus L | -8, 60, 27 | 4.37 | 821 | -/GM | Z=3.51<br>p=0.0004 | OG, r= -0.513*<br>Other, r= -0.037 |
| | | Forceps minor L | -27,51,6 | 3.92 | 727 | -/WM | Z=2.46<br>p=0.01 | OG, r= -0.366*<br>Other, r= -0.013 |
| | Younger Boys | Body of Corpus Callosum | -6, 3, 20 | 3.56 | 896 | +/WM | Z=2.6<br>p=0.009 | YB, r= 0.300*<br>Other, r= -0.069 |

**Note**: Anatomical coordinates (x, y, z) are given in Montreal Neurological Institute (MNI) space. CS = Cluster size; L = Left; OG = Older girls; R = Right; YB = Younger boys. + and - indicate positive and negative associations. Findings overlapping with results of the main analysis are indicated in *italics*. * Denotes significant associations (p<.05).

**Table S7: Post Hoc Analyses Exploring Age Effects Using a Cut-Point of 10 Years, the Mean Peak of Obsessive-Compulsive Disorder Onset [8]**

| Dimension | Group | Brain region | x, y, z | t | CS | Association/ Tissue | Fisher r-to-z | Correlation |
|---|---|---|---|---|---|---|---|---|
| **Age** | | | | | | | | |
| Hoarding | Older | Inferior Frontal Gyrus L | -57, 30, 6 | 4.58 | 725 | +/GM | Z=3.07 p=0.002 | Y, r= -0.043 O, r= 0.339* |
| | | Inferior Frontal Gyrus L | -38, 39, 0 | 5.34 | 1154 | +/WM | Z=2.00 p=0.045 | Y, r= 0.048 O, r= 0.296* |
| **Sex and age** | | | | | | | | |
| Ordering | Younger Boys | Ventral Caudate L | -8, 17, -9 | 3.8 | 549 | -/GM | Z=2.18 p=0.02 | YB, r= -0.347* Other, r= -0.058 |

**Note**: CS = cluster size. *Denotes significant associations (p<.05).

**Table S8: Post Hoc Analyses Exploring Age Effects, in Boys, Using a Cut-Point of 9.5 Years for Boys, the Mean Peak of Obsessive-Compulsive Disorder in Boys [9]**

| Dimension | Group | Brain region | x, y, z | t | CS | Association/ Tissue | Fisher r-to-z | Correlation |
|---|---|---|---|---|---|---|---|---|
| **Sex and age** | | | | | | | | |
| Ordering | Younger Boys | Ventral Caudate L | -15, 6, -14 | 3.52 | 272 | -/GM | Z=1.97 p=0.048 | YB, r= -0.342* Other, r= -0.056 |

**Note:** CS = cluster size; GM = gray matter. *Denotes significant associations (p<.05).

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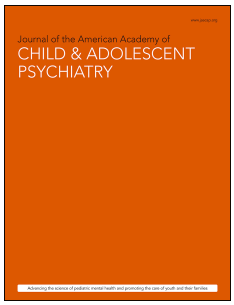
## 4.2 Study 2

**Suñol M**, Saiz-Masvidal C, Contreras-Rodríguez O, Macià D, Martínez-Vilavella G, Martínez-Zalacaín I, Menchón JM, Pujol J, Sunyer J, Soriano-Mas C (2020). Brain Functional Connectivity Correlates of Subclinical Obsessive-Compulsive Symptoms in Healthy Children. J Am Acad Child Adolesc Psychiatry. S0890-8567(20)31836-0. doi: 10.1016/j.jaac.2020.08.435. Online ahead of print.

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RH = OC Symptoms and Brain Connectivity

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Clinical Guidance

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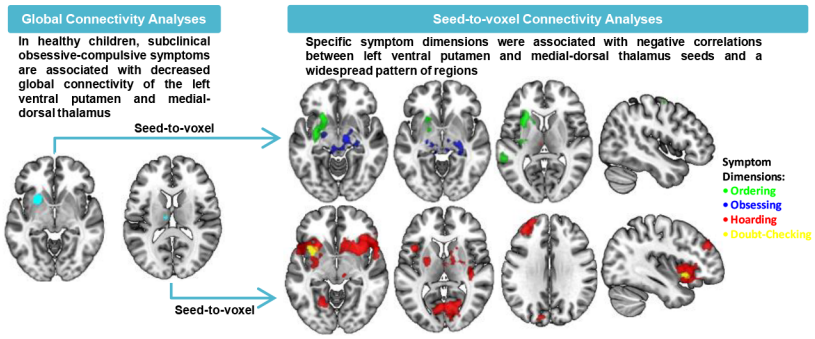

**Abstract**

**Objective**: The commonly observed Subclinical Obsessive-Compulsive (OC) symptoms in healthy children may predispose to Obsessive-Compulsive Disorder (OCD). Therefore, investigating the underlying neurobiology may be relevant to identify alterations in specific brain circuits potentially accounting for clinical heterogeneity in OCD without the confounding effects of clinical samples. Herein, we analyzed the brain correlates of different OC symptoms in a large group of healthy children using functional connectivity measures.

**Method**: We evaluated 227 healthy children (52% girls, mean age±SD=9.71±0.86 years, range 8-12.1). Participants underwent clinical assessment with the Obsessive-Compulsive Inventory-Child Version and a resting-state functional magnetic resonance imaging examination. Total and symptom-specific severity were correlated with voxel-wise global functional connectivity degree values. Significant clusters were then used as seeds of interest in seed-to-voxel analyses. Modulating effects of age and sex were also assessed.

**Results**: Global functional connectivity of the left ventral putamen and medial-dorsal thalamus correlated negatively with total OC severity. Seed-to-voxel analyses revealed specific negative correlations from these clusters with limbic, sensorimotor and insular regions in association with obsessing, ordering and doubt-checking symptoms, respectively. Hoarding symptoms were associated with negative correlations between the left medial-dorsal thalamus and a widespread pattern of regions, being such associations modulated by sex and age.

**Conclusion**: Our findings concur with prevailing neurobiological models of OCD on the importance of cortico-striato-thalamo-cortical (CSTC) dysfunction to account for symptom severity. Notably, we showed that changes in CSTC connectivity are present at subclinical stages, which may result in an increased vulnerability for OCD. Moreover, we mapped different symptom dimensions onto specific CSTC circuit attributes.

**Key words:** obsessive-compulsive disorder; symptom heterogeneity; subclinical symptoms; functional magnetic resonance imaging; functional connectivity

## Introduction

Subclinical obsessive-compulsive (OC) symptoms are fairly common among the general population, with an estimated prevalence between 13%-28% in adults and around 8% in children without a diagnosis of mental health disorder.[1,2] Although these values contrast with a much lower incidence of clinical obsessive-compulsive disorder (OCD), estimated to be between 1-3%,[3] there is evidence to suggest that subclinical OC symptoms precede OCD in a significant percentage of patients,[4] and, more specifically, that the presence of obsessions and compulsions in children increases the likelihood of meeting diagnostic criteria for OCD in adulthood.[1,2]

Significantly, clinical OCD and subclinical OC symptoms are phenomenologically similar, mainly differing in severity and impairment. Therefore, subclinical OC symptoms may be considered part of the OCD spectrum.[1,2] Moreover, the specific symptom profile observed in adults with OCD has been developmentally linked to similar subclinical symptom precursors in childhood,[1] which suggests that subclinical and clinical OC symptoms share common neural signatures that can be studied from a dimensional approach. In a previous study, we evaluated the brain structural features associated with subclinical OC symptoms in healthy children, observing that different symptom dimensions were associated with specific gray and white matter features.[5]

However, clinical and subclinical manifestations of brain disorders seem to be intuitively more closely linked to functional rather than to structural features. For instance, prevailing neurobiological models of OCD emphasizing the role of cortico-striato-thalamic-cortical (CSTC) dysfunction are largely based on functional data,[6] and, more specifically, on brain functional connectivity (FC).[7]

On the other hand, the assessment of the relationships between FC at rest and OC symptom severity has provided relatively heterogeneous findings. Global OC symptom

severity has been associated to reduced connectivity within the limbic (i.e., ventral) CSTC loop,[8] but also to increased FC between the ventral caudate and the OFC,[9,10] or to increased overall connectivity of the orbitofrontal cortex (OFC), putamen, medial prefrontal and anterior cingulate cortices (mPFC and ACC, respectively).[11] Results from studies attempting to link FC features with severity of specific symptom dimensions have provided even more heterogeneous findings, with very little overlap across studies.[10,12]

Importantly, differences in key clinical characteristics of the samples, such as medication use, have been suggested to be an important confounder in these studies.[8] In this regard, studying healthy children with subclinical symptoms may allow for evaluations free from the confounding factors typically observed in clinical samples (related to disorder's chronicity, intensive medication use, or the presence of multiple and severe comorbidities). Nevertheless, this approach causes specific difficulties such as the interaction with rapid neurodevelopmental changes occurring at these ages and the associated between-sex dissimilarities, which may hamper the interpretation of the associations between symptom severity and functional data.[2] Indeed, we observed that sex and age modulated some of the associations between OC symptom dimensions and structural changes in healthy children.[5]

In this study, we aimed at assessing the resting-state FC correlates of symptom severity and symptom profile in a large series of healthy children with a range of subclinical OC symptoms. As in most previous studies, we used a seed-based approach to assess FC from specific brain regions. Nonetheless, since this approach has been criticized because of the need to restrict the analyses to a limited number of pre-selected regions,[13] we opted for an exploratory voxel-wise global connectivity degree assessment in order to identify brain regions with FC patterns significantly associated

with subclinical OC symptoms. Next, we assessed the precise FC patterns from that data-driven identified regions and their associations with specific symptom dimensions. Finally, we evaluated the potential modulatory effects of sex and age. The motivation is two-fold: first, we intend to associate particular OC symptoms and their severity with definite FC features in specific brain circuits while controlling for the most typical confounders of studies with clinical samples. Second, although only longitudinal studies will allow identifying the brain features associated with the development of a full-blown disorder, the results of this study will provide a map of FC features that may eventually be associated with the development of OCD, and, therefore, characterize populations of high-risk individuals.

## Method

### Participants

The sample consisted of 227 healthy school children from the BREATHE project (European Commission: FP7-ERC-2010-AdG, ID 268479). The general project design is described elsewhere.[14] In brief, from an initial sample of 2897 children from 39 schools in the city of Barcelona (Spain), which parents agreed to participate in a study to assess the effects of environmental pollutants on normal neurodevelopment[15-17] (data not used in the present study), 810 families showed interest in participating in a neuroimaging sub-study. Of these, 491 families were successfully contacted by phone, and, from this pool, 263 children completed the imaging protocol. Nevertheless, 10 individuals were excluded based on image quality criteria, while another one was excluded because of incomplete OCI-CV data, leaving a sample of 252 children, which was the sample used to assess the brain structural correlates of subclinical OC

symptoms in a previous publication.[5] In the present study, however, we excluded from the main analyses 25 children with ADHD symptoms, as detailed below, leaving a final sample size of 227 participants.

Importantly, we asked school teachers to, according to the reports of the schools' psycho-pedagogic offices, exclude from this recruitment those children with special needs or mental health, neurological or other major medical conditions (non-severe chronic conditions, like allergies, were not considered an exclusion criterion). However, given the overall prevalence of subthreshold ADHD symptoms[18] and the frequent comorbid presence of these symptoms in children with subclinical OC symptoms,[19] we thought that excluding all children with ADHD symptoms would limit the generalizability of our findings. Therefore, we also asked the school teachers to not exclude children with ADHD symptoms but to indicate, for each child, the presence of these symptoms in a list of 18 items categorized under two separate groups: inattention (nine symptoms) and hyperactivity/impulsivity (nine symptoms). Next, in a later stage of recruitment, those children were interviewed by a mental health clinician of the research team (trained psychologist or psychiatrist), who decided, according to DSM-IV criteria, and taking also into account the information provided by parents or tutors in the Strengths and Difficulties Questionnaire (SDQ),[20] which children fulfilled criteria for ADHD or other diagnoses (considering not only the presence of symptoms, but also their interference in daily activities and their occurrence in multiple contexts). According to these clinical criteria, none of the children fulfilled criteria for ADHD or other mental health diagnoses, although 25 of them had ADHD symptoms. To estimate the modulating effect of ADHD symptoms on our findings, we performed the analyses in the sample of children without ADHD symptoms (n=227, see Results section, below) and in the sample including the with ADHD symptoms (N=252, presented in Figures

S4, S5 and Table S4, available online). All parents or tutors signed the informed consent form approved by the research ethical committee (number 2010/41221/I) of the IMIM-Parc de Salut Mar, Barcelona, and the FP7-ERC-2010-AdG ethics review committee.

Measures

Subclinical OC symptoms were evaluated using the Child Version of the Obsessive-Compulsive Inventory (OCI-CV).[21] The OCI-CV is a self-reported 21-item questionnaire for the assessment of OC symptoms, which consists of 6 different subscales: doubt-checking, obsessing, ordering, hoarding, neutralizing and washing (see Supplement 1, available online).

Imaging Data Acquisition and Preprocessing

Resting-state functional magnetic resonance imaging (rs-fMRI) acquisitions were performed in a 1.5 Tesla Signa Excite system (General Electric, Milwaukee, WI, USA) equipped with an eight-channel phased-array head coil and single-shot echo planar imaging (EPI) software. Despite 3-Tesla exams are nowadays customary in pediatric hospitals, we did not use a 3T magnet following the recommendations of the FP7-ERC Ethics Review Committee that attempted to limit the exposure of healthy children to magnetic fields. Details on the acquisition protocol, which also included a three-dimensional T1-weighted structural acquisition, are reported in Supplement 2, available online, and in Pujol et al. (2016).[16]

Imaging data was transferred and processed on a Microsoft Windows platform running MATLAB version 7.8.0 (The Math Works Inc, Natick, Mass) and CONN Toolbox (Functional Connectivity SPM Toolbox v17; www.nitrc.org/projects/conn).[22] Functional images were preprocessed using the default MNI preprocessing pipeline implemented in

the CONN toolbox, which included realignment and unwrapping, slice-timing correction, and ART-based outlier detection for latter scrubbing (www.nitrc.org/projects/artifact_detect). Structural volumes were segmented and normalized to the MNI space to define gray/white matter and cerebrospinal fluid segments. This segmentation was then applied to functional data, which were finally smoothed with an 8-mm Gaussian kernel. BOLD noise from white matter and cerebrospinal fluid was characterized with the principal component based "aCompCor" method[22] and, in a following denoising step, it was regressed out from BOLD time-series together with the realignment parameters, the motion outliers previously identified in the ART-based outlier detection step, and a linear detrending term. Also, to correct for possible remaining noise, cardiac- and respiration-induced physiological noise were regressed out in first-level analyses. Finally, a band-pass filtering was performed with a frequency window ranging between 0.008Hz-0.09Hz. Importantly, to ensure image quality, sequences were inspected for artifacts before and after every step. Further details about imaging data preprocessing can be found in Supplement 3, available online.



Data Analyses

Descriptive statistics of sociodemographic and psychometric variables were obtained with SPSS 20 (IBM Corp, Armonk, NY). Between-group differences (i.e., between sex and age groups) in these variables were assessed with $x^2$ or independent sample *t*-tests (for qualitative and quantitative variables, respectively), also in SPSS. Likewise, comparisons with a reference sample, were performed with $x^2$ or one-sample t-tests.

*Functional Connectivity Analyses*

In first-level analyses, we used the Intrinsic Connectivity Contrast (ICC) protocol implemented in the CONN toolbox v17. ICC characterizes the strength of the connectivity pattern between each voxel and the rest of the brain by calculating the root mean square of the correlation coefficient values.[23] In second-level analyses, we correlated such ICC values with total and symptom-specific OCI-CV scores. Specifically, we modeled 2 multiple regression models that allowed assessing the effects of the global and symptom-specific OC scores, respectively. The first model evaluated the association between total OCI-CV score and voxel-wise global connectivity, controlling for age, sex and the degrees of freedom from first-level analyses. The second regression model included the different symptom-specific scores from the OCI-CV. Specifically, we included scores with a mean group value higher that 1: doubt-checking, obsessing and ordering and hoarding. These were used as predictor variables, while the other dimensions (i.e., neutralizing and washing, with mean scores<1 and median=0) were included as confounding covariates. Age, sex and degrees of freedom from first level analyses were also included as nuisance variables in this model. Since the effects of predictor variables on voxel values were estimated with the same model (non-independently), significance correction for the number of predictors was not deemed necessary.

The clusters of voxels significantly associated with OCI-CV scores were then used as seeds of interest in post-hoc seed-to-voxel analyses to characterize the regions which correlation with our seeds of interest was associated with OCI-CV total and dimensional scores. In dimensional seed-to-voxel analyses, we restricted our area of interest to a mask encompassing the areas significantly correlated (at $p<0.05$, family-wise error (FWE) corrected) with total OCI-CV score in the seed-based approach, which ultimately limited the likelihood of false positive results. In order to correct for multiple

comparisons across in-mask voxels, statistical significance was set at $p<0.05$, FWE-corrected. Significance threshold was estimated with a voxel-wise nonparametric permutation testing, with 5000 permutations, for which we used the Threshold-Free Cluster Enhancement (TFCE) approach[24] as implemented in the SPM-TFCE toolbox v171 (http://dbm.neuro.uni-jena.de/tfce/).

*Interactions with Sex and Age*

For these analyses, the study sample was divided in two sex and two age subgroups (using a median cut-off point). We extracted the eigenvalues corresponding to the peak values from the above analyses and assessed correlations between these connectivity values and OCI-CV scores within each sex or age subgroup. Next, these correlations were statistically contrasted using a Fisher r-to-z transformation. In addition, interactions between age and OCI-CV were also quantitatively assessed in linear regression analyses. Likewise, in order to explore interactions between sex and age, we also assessed the correlations between connectivity values and OCI-CV scores across younger boys, older boys, younger girls and older girls groups. Significance threshold was set at $p<0.05$.

## Results

Descriptive Variables

OCI-CV scores of our sample were similar to those of a Spanish validation sample,[25] except for obsessing and washing scores, were we obtained lower values (see Table S1, available online). SDQ scores were also lower than those in a normative population.[26] (see Table S2, available online). Clinical interviews detected no cases that were likely

to receive a diagnosis of psychiatric disorder (including OCD), although 25 children (9.92%) had ADHD symptoms (6 were taking methylphenidate hydrochloride or atomoxetine hydrochloride). As mentioned above, we performed the analyses in the sample of children without ADHD symptoms (n=227) and in the whole sample, including children with ADHD symptoms (N=252, presented in Figures S4, S5 and Table S4, available online). Importantly, these two samples did not differ in any sociodemographic or clinical variable. Table 1 presents the demographic and OCI-CV values for the sample without ADHD symptoms, and Table S3, available online, the comparison between the two samples.

Functional Connectivity Analyses

In ICC analyses, we observed significant negative associations between total OCI-CV score and the global connectivity value of the left ventral putamen and the left medial-dorsal thalamus (see Figures 1A and 2A, Table 2 and Figure S1, available online). By contrast, symptom-specific analyses revealed no significant correlations.

Seed-to-voxel analyses from left ventral putamen showed that total OCI-CV scores correlated negatively with a widespread bilateral pattern spanning from ventrolateral prefrontal cortices/frontal opercula and insulae to subcortical striatal, thalamic and limbic regions, and also including premotor and sensorimotor cortices and posterior striate and extrastriate cortices. Results from the left medial-dorsal thalamus seed were similar (see Figure S2 and S3, available online). These patterns were used as masks in subsequent symptom-specific analyses.

Symptom-specific analyses revealed that obsessing symptoms correlated inversely with FC between the left ventral putamen and a limbic cluster encompassing the right hippocampus and the left thalamus and amygdala. Likewise, ordering symptoms also correlated negatively with FC between the left ventral putamen and other striatal

regions (left putamen and pallidum), as well as a sensorimotor cluster including the right precentral gyri and the left Rolandic operculum (extending to left supramarginal and superior temporal gyri). Finally, hoarding symptoms correlated negatively with FC between this same putamen seed and the left medial-dorsal thalamus. These results are presented in Figure 1B and Table 3.

Regarding FC from left medial-dorsal thalamus, we observed that doubt-checking symptoms correlated negatively with FC with the left anterior insula. Hoarding symptoms correlated negatively with FC with a widespread pattern of cortico-subcortical regions, including anterior and posterior brain regions (i.e., the left middle frontal gyrus, the cuneus and the calcarine sulcus) as well as the bilateral striata (including the subthalamic nucleus). These results are presented in Figure 2B and Table 3.

When the analyses were repeated including the children with ADHD symptoms (N=252), we obtained the same pattern of findings, with two additional results: the negative correlation between ordering symptoms and FC of the left ventral putamen also included right striatal regions, and the negative correlation between doubt-checking symptoms and FC of the left medial-dorsal thalamus extended from the left anterior insula to the ventrolateral prefrontal cortex. These results are presented in Figures S4, S5 and Table S4, available online.

Interactions with Sex and Age

Using a median cut-off point of 9.67 years, our sample of 227 children included 116 younger and 111 older children, and 118 girls and 109 boys. Likewise, the interaction between age and sex groups resulted in 62 younger girls, 56 older girls, 54 younger boys, and 55 older boys. Descriptive statistics for age and sex groups are presented in Table S5, available online. Correlations between OCI-CV values and age, as well as

between-sex differences in OCI-CV scores, are presented in Tables S6 and S7, available online. Regarding imaging analyses, we did not observe any significant effect of age on the above findings, in categorical or quantitative analyses. Conversely, we observed a significant effect of sex on the relationship between hoarding scores and FC between the left medial-dorsal thalamus and the subthalamic nucleus. Specifically, this correlation was significantly more negative in girls (girls, r=-0.342; boys, r=-0.082; Z=2.04, p=0.041). Likewise, the assessment of the interaction between sex and age showed that the association between hoarding traits and FC between the medial-dorsal thalamic seed and the cluster encompassing posterior cortical regions (i.e., cuneus and calcarine sulcus), was significantly more negative in the group of older girls (older girls, r=-0.507; other groups, r=-0.112; Z=2.83, p=0.004). These results were also observed with the whole sample of children (N=252).

**Discussion**

In this study, we assessed, for the first time in the literature, the correlation between subclinical OC symptoms and whole-brain FC in a large sample of healthy children. Remarkably, despite employing a whole-brain exploratory approach, our results showed that subclinical OC symptoms were related to FC variations in the ventral putamen and the medial-dorsal thalamus, two relay nodes of the CSTC circuits[27] which dysfunction, according to prevailing neurobiological accounts,[6] is thought to underlie expression of OC symptoms. Our findings not only significantly reinforce neurobiological models of OCD, but also indicate that such alterations are already present at subclinical, or preclinical, disease stages, and do not depend on confounders typical of adult clinical samples. Moreover, we also obtained a set of findings showing specific associations between distinct patterns of FC from these two CSTC relay nodes and specific symptom

subtypes, what should critically contribute to describe clinical heterogeneity in OCD from a neurobiological perspective.

According to current formulations of CSTC circuit organization,[28] the ventral putamen is the main subcortical relay of the ventral cognitive circuit, linking the ventrolateral prefrontal cortex with the thalamus. This circuit is involved in response inhibition and cognitive control of emotions, functions known to be altered in individuals with OCD.[6,28] The inverse correlation reported here between left ventral putamen global connectivity and OC symptom severity may be therefore suggestive of a decreased crosstalk between neighboring regions of the ventral cognitive circuit in children with OC symptoms. Such interpretation concurs with previous research in clinical children and adult OCD samples showing decreased resting-state FC within the ventral cognitive circuit,[9,29] as well as with studies in adult research participants with OCD reporting negative correlations between Y-BOCS scores and resting-state activity in the left ventral putamen.[30]

The medial-dorsal thalamus, on the other hand, acts as the final relay point of different CSTC circuits before reaching the prefrontal cortex. Consequently, it is difficult to establish a direct association between findings in this region and a particular CSTC circuit. The progressive compression of fibers from the different CSTC circuits within the same nuclei[27] makes it impossible, with the resolution of current imaging techniques, to elucidate whether our findings stem from decreased connectivity in a specific CSTC circuit or from more pervasive FC alterations involving all the different circuits reported to be altered in OCD (i.e., dorsal cognitive, ventral cognitive and affective circuits).[28] In any case, our results agree with previous studies reporting thalamic alterations in research participants with OCD, especially in pediatric populations, although mainly at the structural level.[31,32]

Obsessing symptoms correlated negatively with FC between left ventral putamen and a limbic cluster encompassing the hippocampus, the amygdala and the ventral thalamus. Overall decreased FC of limbic regions (i.e., parahippocampus, amygdala) has been observed in OCD,[33] and, more specifically, previous research from our group showed that aggression symptoms, which, together with sexual/religious symptoms have been grouped together under the umbrella of unacceptable thoughts or pure obsessions,[34] predicted decreased connectivity between bilateral amygdala and ventral striatum.[10] Nevertheless, in that study, connectivity alterations were located in the ventral caudate (which is supposed to be part of the affective CSTC loop), and connectivity from the ventral putamen was not assessed. Combining that results with present ones, it may be suggested that subjects with aggressive or obsessive symptoms may show a distinct FC decrease between limbic regions and the ventral striatum, probably including ventral caudate and ventral putamen relay nodes. Such decreased limbic-ventral striatal connectivity is likely to account for fear-driven symptomatology, including heightened amygdala response in emotional processing, which has been specifically observed in research participants with aggressive or sexual/religious symptoms.[35]

Ordering traits correlated negatively with FC between the left ventral putamen and other subcortical areas (including the sensorimotor dorsal striatum), as well as with sensorimotor cortices (right precentral gyrus and left Rolandic operculum, extending to supramarginal and superior temporal gyri). Previous studies have reported decreased FC in sensorimotor regions in individuals with OCD,[8,9,36] which is consistent with accounts suggesting that poor control over repetitive behaviors OCD may relate to impairments in sensorimotor gating and prepulse inhibition.[37] The association between impaired connectivity of sensorimotor regions and ordering symptoms is also in agreement with extant literature, since abnormalities in sensorimotor regions have been associated with

ordering in OCD samples[38] and with the presence of sensory phenomena,[39] a symptom that is frequently observed in research participants with symmetry/ordering symptoms.[39] Increased prevalence of symmetry/ordering symptoms has also been consistently observed in motor tic disorders.[38]

Doubt-checking traits correlated negatively with FC between left medial-dorsal thalamus and left anterior insula. Concurring with our findings, in adult OCD samples, decreased connectivity between these structures has been associated with increased overall severity.[40] More specifically, it has been reported that research participants with predominant checking symptoms display a high degree of intolerance to uncertainty, which has been linked to abnormal insula activation.[41] As part of the salience network, alterations in connectivity of the anterior insula may impair the correct evaluation of salient stimuli in uncertain environments,[42] and this could potentially lead to an abnormally increased emotional salience of stimuli. Speculatively, compulsive checking might be triggered to attenuate the increased anxiety levels stemming from functional impairments in the salience network. In agreement with such notions, insular volume alterations are more significant in research participants with predominant checking symptoms.[42] Likewise, alterations in the connectivity between the thalamic seed and this area, putatively involving the ventral cognitive CSTC circuit, could underpin deficits in cognitive flexibility, which are fairly common in both clinical and subclinical samples with checking symptoms.[43,44]

Notably, when including children with ADHD symptoms, we observed additional significant clusters in these two last sets of findings. These clusters involved right subcortical regions (significantly connected to the left ventral putamen in the analysis with ordering symptom) and the left ventrolateral prefrontal cortex (significantly connected to the left medial-dorsal thalamus in the analysis with doubt-checking

symptoms). This is in agreement with recent research indicating that activations in these regions during different protocols may discriminate between children with OCD and ADHD.[45,46] The role of ADHD symptoms should therefore be considered when interpreting alterations in FC of left ventral putamen and medial-dorsal thalamus with these structures in OCD samples.

Finally, hoarding was the only group of symptoms correlating with FC decreases between our two main regions of interest (the ventral putamen and the dorsal-medial thalamus), which may be suggestive of a disrupted information flow within the ventral cognitive circuit. However, the pattern that emerged when assessing the association between hoarding symptoms and left medial-dorsal thalamus connectivity included also striatal areas outside the ventral putamen, indicating a pervasive alteration of CSTC connectivity across different circuits. Notably, a recent study found decreased connectivity between the bilateral caudate and the thalamus in research participants with hoarding disorder,[47] although we did not observe a specific involvement of caudate nuclei. Further research is warranted to elucidate the extent of CSTC FC alterations in individuals with hoarding symptoms and the putative differences between subjects with OCD and hoarding symptoms (even at subclinical stages) and pure hoarders. Likewise, the potential interactions with sex of such hoarding-related alterations should be also further investigated, since we observed that the relationship between hoarding symptoms and FC with the subthalamic nucleus, a major relay in the indirect path of CSTC circuits,[27] was more negative in girls.

Hoarding symptoms were also negatively correlated with FC between the thalamic seed and dorsolateral prefrontal and medial occipital cortices. Alterations in FC involving dorsolateral prefrontal cortex areas seem to reinforce the idea of a pervasive alteration in CSTC circuits in research participants with hoarding symptoms (i.e., also involving

dorsal cognitive circuits), and may likely account for the deficits in executive functioning characterizing these individuals.[47] Regarding correlations between hoarding symptoms and FC decrease with medial occipital areas, it is important to note that these were significantly more negative in older girls. This finding, together with the above-mentioned interaction with sex involving subthalamic connectivity, suggests that female individuals, especially during pre-adolescence, may show an increased susceptibility to hoarding symptoms. Consistent with this, it has been shown that female research participants with hoarding behavior experience more severe OC symptoms.[48] Similarly, in terms of heritability, hoarding symptoms are more likely to be shared between mothers and daughters than between other parental-sibling dyads.[48] However, the relative weight of genetics and environment on hoarding symptoms seems to vary over time, potentially, in interaction with sex. Thus, in a twin study, it was shown that environmental factors significantly contributed to the presence of hoarding traits in 15-year-old girls, but not in other age groups.[49] Such environmental factors included parental style, with female individuals with OCD and hoarding traits reporting lower scores of maternal care than their male counterparts.[50] Although we acknowledge the difficulty of associating FC alterations between the thalamus and the medial occipital cortex with specific psychological processes triggering or worsening hoarding symptoms, the results presented here are probably the first neurobiological correlate of the link reported at the clinical and epidemiological level between hoarding symptoms and female sex during adolescence. Nevertheless, these results should be interpreted with caution since we did not assess pubertal status of participants and, therefore, could not distinguish between child and adolescent participants.

Since the presence of OC symptoms in children increases the risk of developing a full-blown disorder,[1] the neurobiological features described here may tentatively be

considered as potential neuroimaging markers of increased risk for OCD. Nevertheless, longitudinal designs with follow-up clinical evaluations will be needed to discriminate between neurobiological features predicting later development of OCD and those exclusively associated with subclinical symptoms. Besides, adding follow-up imaging assessments would also allow inferring causality between brain and clinical changes. Other limitations of the present study include the lack of a group of an age- and sex-matched group of research participants diagnosed with OCD. Such comparison would allow confirming whether neurobiological features associated here with OC symptoms are also present in clinical samples. Furthermore, despite exclusively recruiting healthy participants, there are potential confounders than have not been completely controlled for, such as familiar history of psychiatric or neurological conditions, or intake of non-psychiatric medicines. Likewise, since each participant was interviewed by one single clinician, we did not obtain intra-rater reliability measurements to validate study inclusion. Finally, we assessed resting-state FC, but other functional approaches could have been equally used. In this regard, however, resting-state FC assessments prevent the variability induced by different levels of performance in task-based fMRI while capturing the network-level alterations that characterize OCD.[7]

In conclusion, our results are supportive of the role of CSTC circuit dysfunction in OC symptom manifestations, even at subclinical stages. Moreover, we also suggest that clinical heterogeneity of the disorder may stem from changes in specific CSTC circuits. Specifically, FC alterations between CSTC relay nodes and limbic, sensorimotor and insular regions were associated with obsessing, ordering and doubt-checking symptoms, respectively, while hoarding symptoms were associated with a more widespread pattern of alteration. Such patterns of disrupted FC may be eventually used as imaging biomarkers of increased risk for OCD.

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**Table 1. Sociodemographic Information and Obsessive-Compulsive Inventory-Child Version (OCI-CV) Scores for the Study Sample**

| **Sample characteristics (n= 227)** | | |
|---|---|---|
| | *Mean (SD)* | *Median* |
| Age (years) | 9.71 (0.86)<br>Range: 8-12.1 | 9.67 |
| Sex | | |
| Girls, n(%) | 118 (52%) | |
| Boys, n(%) | 109 (48%) | |
| Non-Psychiatric Medication, n(%) | 19 (8.4%)[a] | |
| School Performance (0-5) | 3.84 (0.98) | 4 |
| Mother's Education (0-5) | 4.56 (0.78) | 5 |
| **OCI-CV scores (n= 227)** | | |
| | *Mean (SD)* | *Median* |
| Doubting-Checking | 2.25 (1.52) | 2 |
| Hoarding | 2.04 (1.26) | 2 |
| Neutralizing | 0.38 (0.72) | 0 |
| Obsessing | 1.59 (1.40) | 1 |
| Ordering | 2.23 (1.60) | 2 |
| Washing | 0.59 (0.82) | 0 |
| Total | 9.08 (4.08) | 9 |

Note: [a] Non-Psychiatric medication included acetaminophen, antihistamines or antibiotics

**Table 2. Associations Between Specific Obsessive-Compulsive Dimension Scores and the Intrinsic Connectivity Contrast Values**

| OC Dimension | Brain Region | x, y, z | TFCE | CS |
|---|---|---|---|---|
| Total OC traits | ventral putamen L | -30, 6, -12 | 916.26 | 887 |
| | medial dorsal thalamus L | -8, -16, 14 | 567.12 | 13 |

Note: Anatomic coordinates (x, y, z) are given in Montreal Neurological Institute space. CS = cluster size in voxels; L=Left; OC=o bsessive-compulsive; TFCE = threshold-free cluster enhancement.

**Table 3. Associations Between Specific Obsessive-Compulsive Dimension Scores and the Seed-Based Functional Connectivity**

| OC Dimension | Seed | Brain Region | x, y, z | TFCE | CS |
|---|---|---|---|---|---|
| Obsessing | ventral putamen L | hippocampus R | 22, -20, -6 | 221.60 | 536 |
| | | thalamus L | -20, -20, -2 | | |
| | | amygdala L | -22, -8, -12 | | |
| Ordering | ventral putamen L | putamen L | -20, 16, -10 | 545.24 | 2528 |
| | | rolandic operculum – superior temporal gyrus L | -60, -42, 10 | | |
| | | supramarginal L | -52, -28, 30 | | |
| | | precentral R | 54, -10, 46 | 306.09 | 12 |
| Hoarding | ventral putamen L | medial dorsal thalamus L | -6, -20, 10 | 288.32 | 17 |
| | medial dorsal thalamus L | putamen R | 26, 8, 4 | 718.79 | 2558 |
| | | subthalamic nucleus R | 10, -16, -6 | | |
| | | medial dorsal thalamus L | -8, -20, 14 | | |
| | | cuneus – calcarine | 10, -86, 10 | 575.26 | 1647 |
| | | putamen L | -32, -2, -2 | 559.04 | 2155 |
| | | middle frontal gyrus L | -34, 44, 32 | 540.43 | 632 |
| Doubt-Checking | medial dorsal thalamus L | insula L | -36, 14, -6 | 438.14 | 138 |

Note: Anatomic coordinates (x, y, z) are given in Montreal Neurological Institute space. CS = cluster size in voxels; L = left; OC = obsessive-compulsive; R = right; TFCE = threshold-free cluster enhancement.

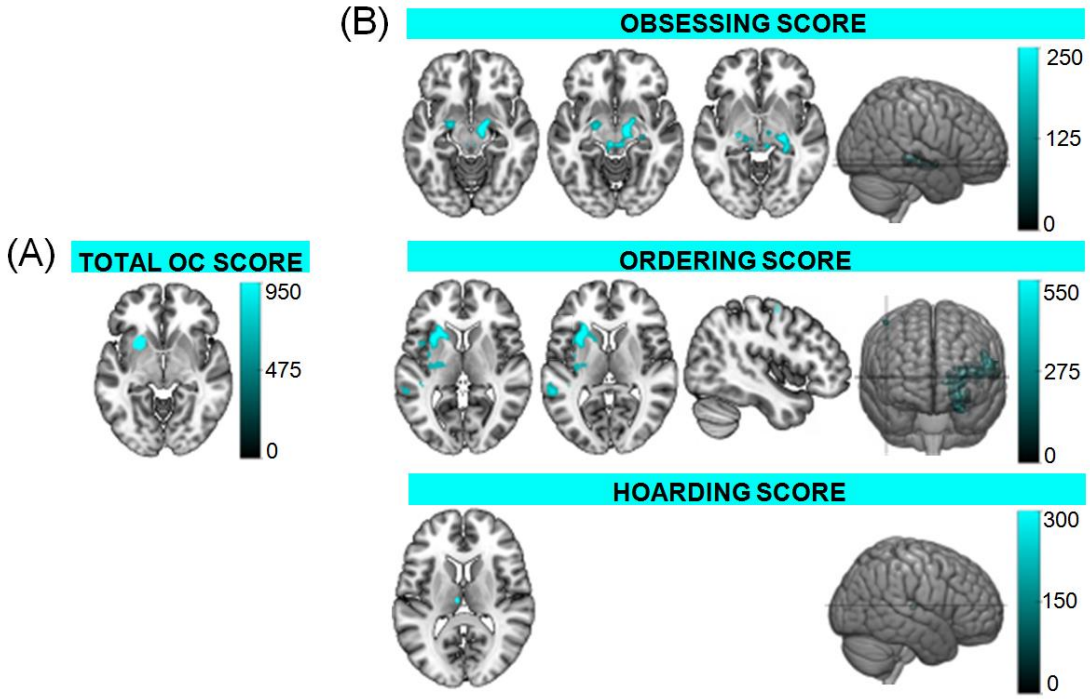


**Figure 1: Results From Functional Connectivity Analyses**

Note: **(A)** In an Intrinsic Connectivity Contrast analysis, total severity of subclinical Obsessive-Compulsive (OC) symptoms showed a negative correlation with global connectivity of the left ventral putamen. No significant correlations were observed in symptom specific analyses. **(B)** Conversely, in seed-based symptom specific analyses, we observed that the functional connectivity pattern of the left ventral putamen correlated negatively with the severity of obsessing, ordering and hoarding symptoms. Color bars indicate Threshold-Free Cluster Enhancement values. Images are displayed in neurological convention.

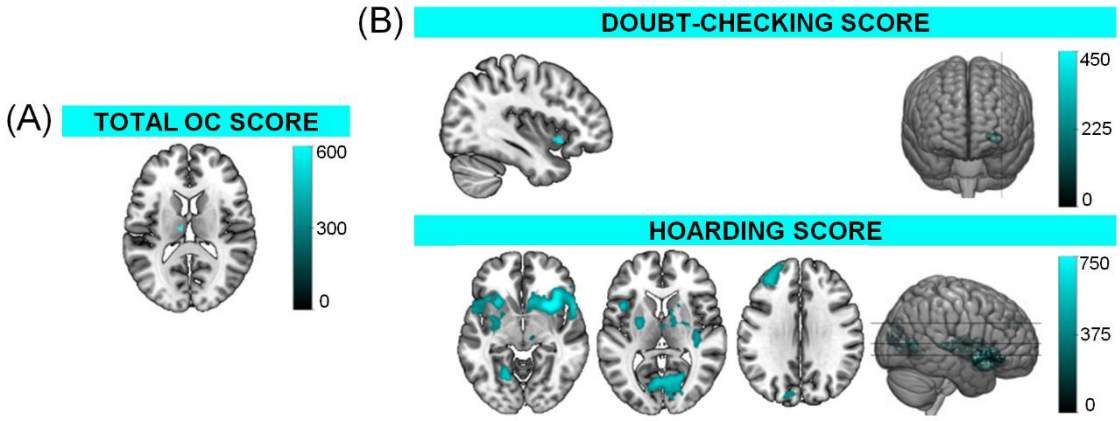


**Figure 2: Results From Functional Connectivity Analyses**

Note: **(A)** In an Intrinsic Connectivity Contrast analysis, total severity of subclinical Obsessive-Compulsive (OC) symptoms showed a negative correlation with global connectivity of the left medial-dorsal thalamus. No significant correlations were observed in symptom specific analyses. **(B)** Conversely, in seed-based symptom specific analyses, we observed that the functional connectivity pattern of the left medial-dorsal thalamus correlated negatively with the severity of doubt-checking and hoarding symptoms. Color bars indicate Threshold-Free Cluster Enhancement values. Images are displayed in neurological convention.

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**Conflict of Interest:**

Drs. Contreras-Rodríguez, Macià, Pujol, Sunyer, Menchón, Soriano-Mas, Mss. Suñol, Saiz-Masvidal, Messrs. Martínez-Vilavella, and Martínez-Zalacaín report no biomedical financial interests or potential conflicts of interest.

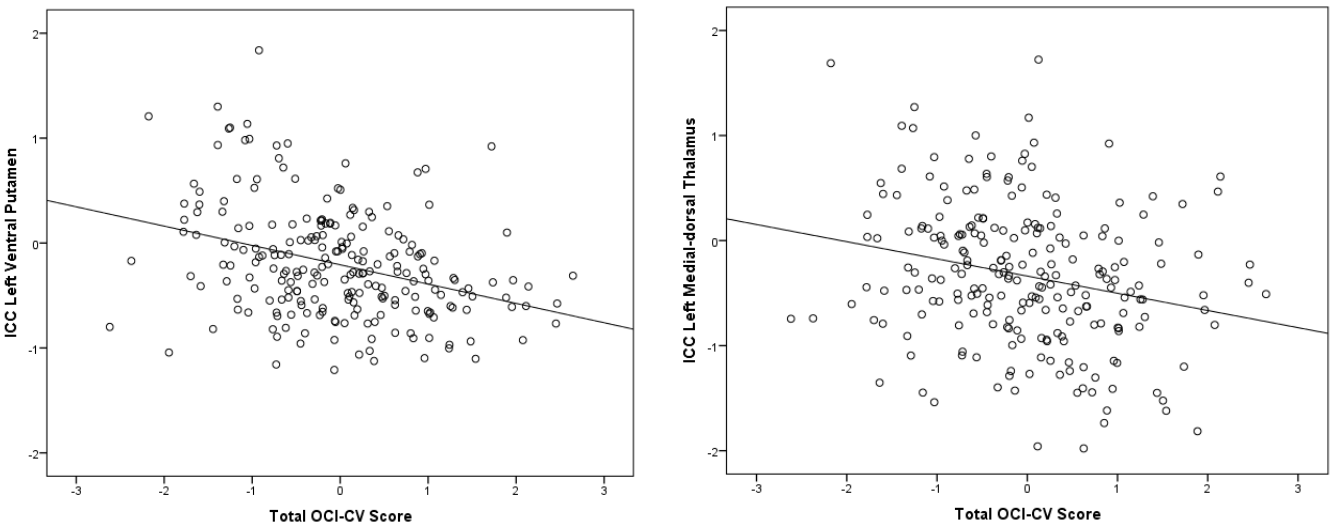


**Figure S1:** Scatter plots depicting the correlations between total Obsessive Compulsive Inventory-Child Version (OCI-CV) scores and the global connectivity values of the left ventral putamen (left) and left medial-dorsal thalamus (right), derived from Intrinsic Connectivity Contrast (ICC) analyses. Values were adjusted for age, sex and degrees of freedom from first level analyses.

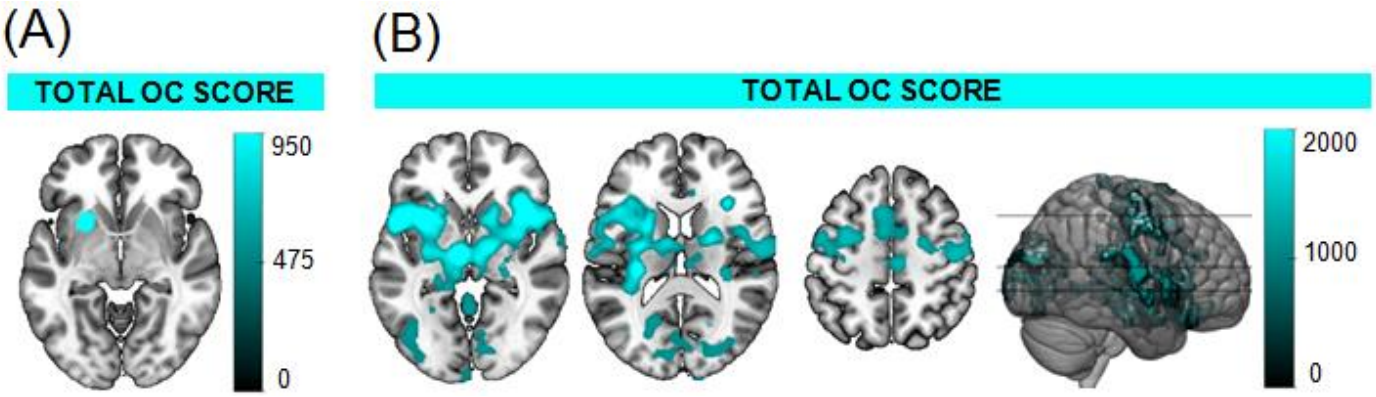


**Figure S2:** Results from functional connectivity analyses. **(A)** Location of the left ventral putamen seed derived from Intrinsic Connectivity Contrast analysis. **(B)** In a seed-based analysis, the voxel-wise pattern of functional connectivity from this seed showed a negative correlation with total severity of subclinical Obsessive-Compulsive (OC) symptoms. Color bars indicate Threshold-Free Cluster Enhancement values. Images are displayed in neurological convention.

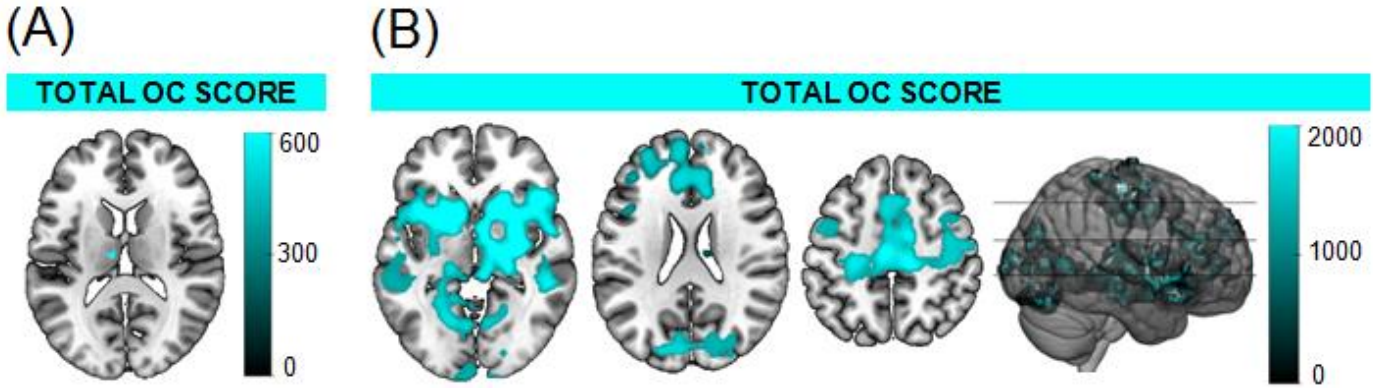


**Figure S3:** Results from functional connectivity analyses. **(A)** Location of the left medial-dorsal thalamus seed derived from Intrinsic Connectivity Contrast analysis. **(B)** In a seed-based analysis, the voxel-wise pattern of functional connectivity from this seed showed a negative correlation with total severity of subclinical Obsessive-Compulsive (OC) symptoms. Color bars indicate Threshold-Free Cluster Enhancement values. Images are displayed in neurological convention.

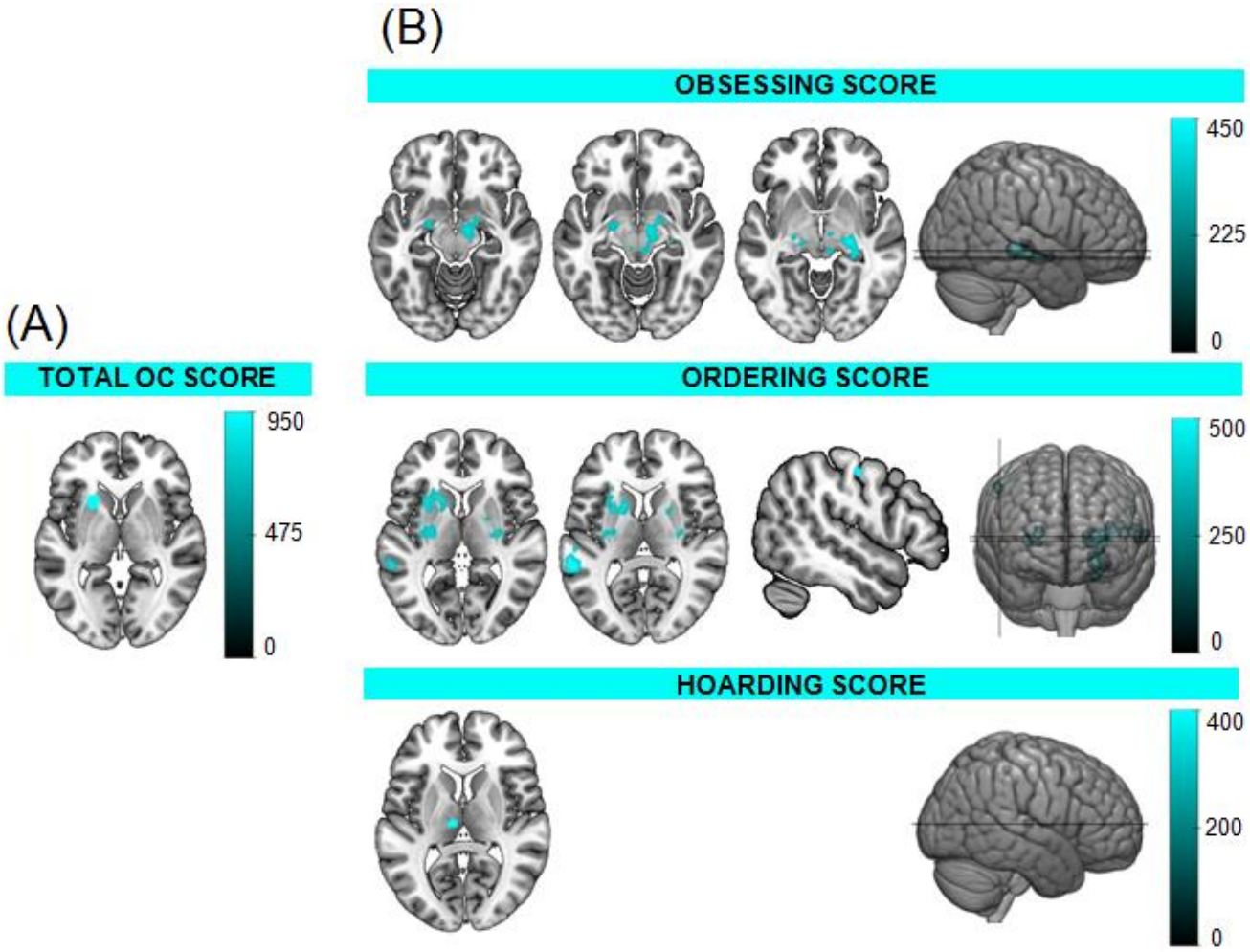


**Figure S4:** Results from functional connectivity analyses including the children with Attention-Deficit/Hyperactivity Disorder (ADHD) symptoms (N=252). **(A)** In an Intrinsic Connectivity Contrast analysis, total severity of subclinical Obsessive-Compulsive (OC) symptoms showed a negative correlation with global connectivity of the left ventral putamen. No significant correlations were observed in symptom specific analyses. **(B)** Conversely, in seed-based symptom specific analyses, we observed that the functional connectivity pattern of the left ventral putamen correlated negatively with the severity of obsessing, ordering and hoarding symptoms. Color bars indicate Threshold-Free Cluster Enhancement values. Images are displayed in neurological convention.

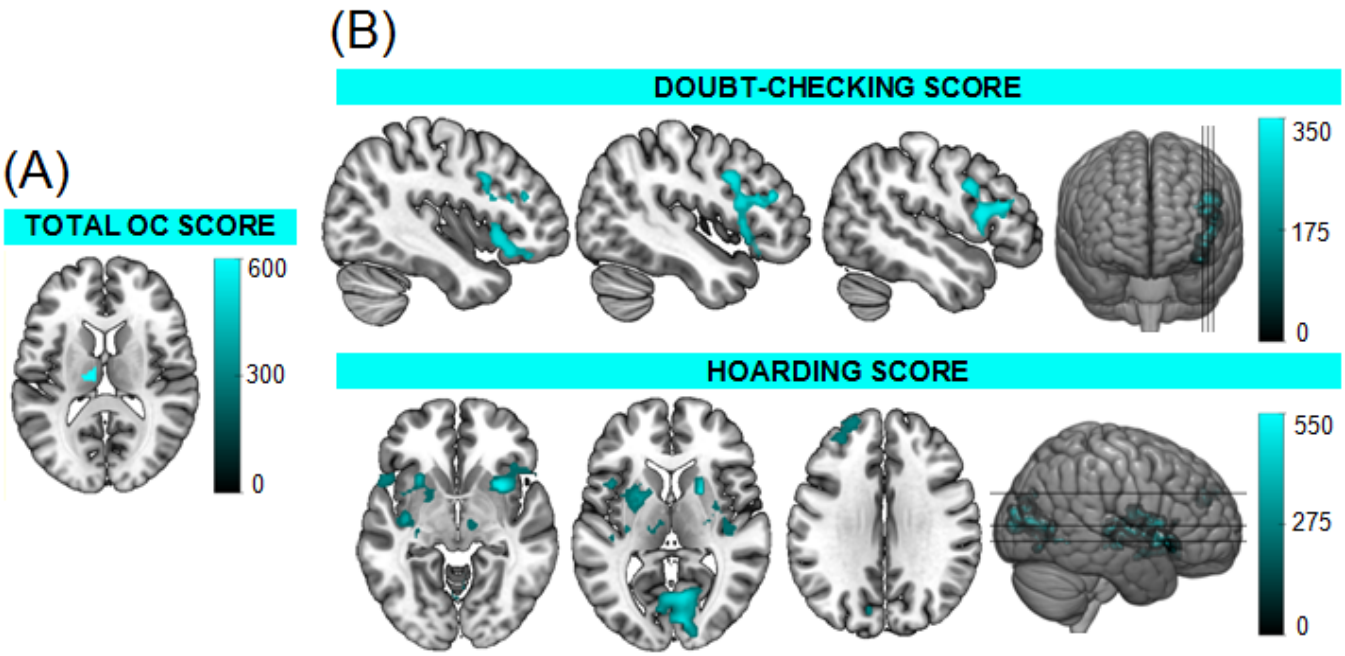


**Figure S5:** Results from functional connectivity analyses including the children with Attention-Deficit/Hyperactivity Disorder (ADHD) symptoms (N=252). **(A)** In an Intrinsic Connectivity Contrast analysis, total severity of subclinical Obsessive-Compulsive (OC) symptoms showed a negative correlation with global connectivity of the left medial-dorsal thalamus. No significant correlations were observed in symptom specific analyses. **(B)** Conversely, in seed-based symptom specific analyses, we observed that the functional connectivity pattern of the left medial-dorsal thalamus correlated negatively with the severity of doubt-checking and hoarding symptoms. Color bars indicate Threshold-Free Cluster Enhancement values. Images are displayed in neurological convention.

**Supplement 1:**

**THE OBSESSIVE COMPULSIVE INVENTORY-CHILD VERSION (OCI-CV)**

Responses in the OCI-CV are provided in a 3-point Likert-type scale (0=*never* - 2=*always*) to increase sensitivity beyond simple yes/no responses. The OCI-CV has shown good internal consistency with $\alpha \geq 0.81$ for the total score and each of the subscales. Test-retest reliability coefficients are 0.77 for the total scale, and range from 0.68 to 0.89 for the subscales. Correlations between OCI-CV and the gold-standard measures of pediatric OCD, such as the CY-BOCS and the NIMH Global Obsessive Compulsive Scale, are statistically significant and moderate.[1]

This questionnaire was considered optimal for the study purposes because it can be administered to healthy subjects to assess subclinical symptoms, covers a wide age range (7 to 17), provides severity scores for six common OCD symptom dimensions, and it is easy for children populations to understand and relatively quick to complete.

The OCI-CV assesses six different symptom domains:

**Doubting-Checking**: One of the most common dimensions of OCD. It is characterized by intolerance of uncertainty that leads to excessive worry and checking compulsions. OCI-CV assesses this dimension with the following items: "*I check many things over and over again*", "*After I have done things, I'm not sure if I really did them*", "*Even after I'm done I still worry that I didn't finish things*", "*I check doors, windows, and drawers over and over again*" and "*Even when I do something very carefully I don't think I did it right*".

**Hoarding**: Characterized by obsessions and compulsions related to hoarding unnecessary objects. OCI-CV assesses this dimension with the following items: "*I collect so much stuff that it gets in the way*", "*I collect things I don't really need*" and "*I don't throw things away because I'm afraid I might need them later*".

**Neutralizing**: Involves counting and repeating compulsions generally associated with numbers. OCI-CV assesses this dimension with the following items: "*I need to count while I do things*", "*I get behind in my school-work because I repeat things over and over again*" and "*I have to say some numbers over and over*".

**Obsessing**: Involves intrusive thoughts that cause distress and fear. OCI-CV assesses this dimension with the following items: "*I think about bad things and can't stop*", "*I'm upset by bad thoughts*", "*I get upset by bad*

*thoughts that pop into my head when I don't want them to*" and "*If a bad thought comes into my head, I need to say certain things over and over*".

**Ordering**: Characterized by the presence of obsessions about symmetry or "just right" perceptions and ordering compulsions. OCI-CV assesses this dimension with the following items: "*I get upset if my stuff is not in the right order*", "*I get upset if people change the way I arrange things*" and "*I need things to be in a certain way*".

**Washing**: Characterized by the presence of contamination obsessions that are counteracted with compulsive and excessive cleaning. OCI-CV assesses this dimension with the following items: "*I feel like I must wash and clean over and over again*", "*I worry a lot about things being clean*" and "*I wash my hands more than other kids*".

**References:**

1. Foa EB, Coles M, Huppert JD, Pasupuleti RV, Franklin ME, March J. Development and Validation of a Child Version of the Obsessive Compulsive Inventory. *Behav Ther*. 2010;41(1):121-132.

**Supplement 2:**

**ACQUISITION PROTOCOL**

The rs-fMRI sequences consisted of gradient recalled acquisition in the steady-state with repetition time 2000 ms; echo time 50 ms; pulse angle 90°; field of view 24 cm; 64 × 64-pixel matrix; slice thickness 4 mm (inter-slice gap, 1.5 mm). Twenty-two interleaved slices were prescribed parallel to the anterior–posterior commissure line covering the entire brain. For each participant, we acquired 180 whole-brain EPI volumes, for a total duration of 6 minutes. Furthermore, the first four (additional) images were discarded to allow magnetization to reach equilibrium. During the rs-fMRI, children were instructed to relax, stay awake and lie still without moving while keeping their eyes closed. High-resolution 3D anatomical images were also obtained using an axial T1-weighted 3D fast spoiled gradient inversion recovery-prepared sequence. A total of 134 contiguous slices were acquired with inversion time 400 ms; repetition time 11.9 ms; echo time 4.2 ms; flip angle 150; field of view 30 cm; 256 × 256-pixel matrix; slice thickness 1.2 mm.

**Supplement 3:**

## IMAGING DATA EXCLUSION CRITERIA

Imaging data were first reviewed by an expert neuroradiologist to detect clinically relevant and gross anatomical abnormalities from T1 weighted images. Second, in the course of image conversion and pre-processing, a trained operator reviewed all functional scans to detect the presence of excessive movement, acquisition artifacts (such as magnetic field inhomogeneities) and image processing artifacts (e.g., problems with realignment or normalization algorithms). From the 263 children who underwent the imaging protocol 2 had a gross structural anomaly, 1 had an acquisition artifact, and 7 were removed due to excessive movement. Movement was corrected with the ART-based outlier detection and scrubbing method,[1] and participants with less than 4 minutes of non-censored data were excluded from the study because of excessive movement. Conversely, in subjects with more than 4 minutes of non-censored data, outlier volumes were regressed out during the denoising step. Importantly, to compensate for the different across-subject data acquisition points, in second level analyses we covaried for the degrees of freedom from first-level analyses.

**References:**

1. Parkes L, Fulcher B, Yücel M, Fornito A. An evaluation of the efficacy, reliability, and sensitivity of motion correction strategies for resting-state functional MRI. *Neuroimage*. 2018;171:415-436.

**Table S1: COMPARISON OF OBSESSIVE COMPULSIVE INVENTORY-CHILD VERSION SCORES BETWEEN THE STUDY SAMPLE AND THE SPANISH VALIDATION SAMPLE [1]**

| Obsessive Compulsive Inventory-Child Version (OCI-CV) | | |
|---|---|---|
| **Our project** | **N=252 (8-12y)** | |
| | *Mean (SD)* | *Median* |
| Doubting-Checking | 2.30 (1.52) | 2 |
| Hoarding | 2.05 (1.29) | 2 |
| Neutralizing | 0.40 (0.74) | 0 |
| Obsessing | 1.56 (1.40) * | 1 |
| Ordering | 2.26 (1.61) | 2 |
| Washing | 0.64 (0.87) * | 0 |
| Total | 9.21 (4.19) * | 9 |
| **Spanish Validation Sample** | **N=914 (8-18y)** | |
| | *Mean (SD)* | *Median* |
| Doubting-Checking | 2.13 (1.64) | - |
| Hoarding | 1.90 (1.35) | - |
| Neutralizing | 0.32 (0.72) | - |
| Obsessing | 2.39 (2.01) | - |
| Ordering | 2.20 (1.68) | - |
| Washing | 3.10 (2.16) | - |
| Total | 11.34 (6.09) | - |

Note: *Denotes a score significantly different from the score of the Spanish validation sample ($p<0.05$, one-sample t-test).

**References:**

1. Rosa-Alcázar AI, Ruiz-García B, Iniesta-Sepúlveda M, López-Pina JA, Rosa-Alcázar Á, Parada-Navas JL. Obsessive Compulsive Inventory-Child Version (OCI-CV) in a Spanish community simple of children and adolescents. *Psicothema*. 2014;26(2):174-179.

**Table S2: COMPARISON OF STRENGTHS AND DIFFICULTIES QUESTIONNAIRE SCORES BETWEEN THE STUDY SAMPLE AND THE SPANISH VALIDATION SAMPLE [1]**

| Strengths and Difficulties Questionnaire (SDQ) | | | | | | |
|---|---|---|---|---|---|---|
| **Our project** | **Total N=247 (8-12y)** | | **Girls n=121** | | **Boys n=126** | |
| | *Mean (SD)* | *Median* | *Mean (SD)* | *Median* | *Mean (SD)* | *Median* |
| Emotional Symptoms (0-10) | 1.95 (1.79) * | 2 | 1.96 (1.77) * | 2 | 1.94 (1.81) | 1 |
| Conduct Problems (0-10) | 1.74 (1.66) * | 1 | 1.44 (1.42) * | 1 | 2.02 (1.83) * | 2 |
| Hyperactivity/Inattention (0-10) | 3.94 (2.48) * | 4 | 3.57 (2.27) * | 4 | 4.30 (2.63) | 4 |
| Peer Relationship Problems (0-10) | 1.21 (1.49) * | 1 | 1.07 (1.35) * | 1 | 1.35 (1.61) | 1 |
| Total Difficulties Score (0-40) | 8.84 (5.31) * | 8 | 8.03 (4.85) * | 8 | 9.61 (5.64) | 8 |
| Prosocial Behavior (0-10) | 8.64 (1.58) * | 9 | 8.92 (1.37) * | 9 | 8.37 (1.73) * | 9 |
| **Spanish Validation Sample** | **Total N=6266 (34.10% aged 8-11y)** | | **48.59% Girls** | | **51.41% Boys** | |
| | *Mean (SD)* | *Median* | *Mean (SD)* | *Median* | *Mean (SD)* | *Median* |
| Emotional Symptoms (0-10) | 2.10 (0.06) | 2 | 2.19 (0.08) | 2 | 2.00 (0.08) | 2 |
| Conduct Problems (0-10) | 1.87 (0.05) | 1 | 1.83 (0.07) | 1 | 1.91 (0.07) | 1 |
| Hyperactivity/Inattention (0-10) | 4.19 (0.08) | 4 | 3.97 (0.12) | 4 | 4.39 (0.11) | 5 |
| Peer Relationship Problems (0-10) | 1.32 (0.05) | 1 | 1.33 (0.06) | 1 | 1.31 (0.06) | 1 |
| Total Difficulties Score (0-40) | 9.49 (0.18) | 9 | 9.34 (0.26) | 8 | 9.63 (0.25) | 9 |
| Prosocial Behavior (0-10) | 8.81 (0.04) | 10 | 9.04 (0.05) | 10 | 8.59 (0.07) | 9 |

Note: *Denotes a score significantly different from the score of the Spanish validation sample ($p<0.05$, one-sample t-test).

**References:**

1. Barriuso-Lapresa LM, Hernando-Arizaleta L, Rajmil L. Reference values of the Strengths and Difficulties Questionnaire (SDQ) version for parents in the Spanish population, 2006. *Actas Esp Psiquiatr*. 2014;42(2):43-48.

**Table S3: COMPARISON OF SOCIODEMOGRAPHIC AND CLINICAL VARIABLES BETWEEN THE STUDY SAMPLE (n=227) AND THE SAMPLE INCLUDING THE CHILDREN WITH ATTENTION-DEFICIT/HYPERACTIVITY DISORDER SYMPTOMS (N=252)**

| **Sample Characteristics** | **Study Sample (n= 227)** | **Sample including children with ADHD symptoms (N=252)** | **Statistics** | |
|---|---|---|---|---|
| | *Mean (SD)* | *Mean (SD)* | $t/x^{2\wedge}$ | *p* |
| Age (years) | 9.71 (0.86) | 9.72 (0.87) | 0.09 | 0.93 |
| Sex | | | 0.70 | 0.40 |
| Girls, n(%) | 118 (52%) | 124 (49.2%) | | |
| Boys, n(%) | 109 (48%) | 128 (50.8%) | | |
| Non-Psychiatric Medication | 21 (8.4%) | 19 (8.4%) | 0.00 | 0.99 |
| School Performance (0-5) | 3.84(0.98) | 3.72(1.04) | 1.81 | 0.07 |
| Mother's Education (0-5) | 4.56 (0.78) | 4.53 (0.81) | 0.58 | 0.56 |
| **OCI-CV scores** | **n= 227** | **N=252** | | |
| Doubting-Checking | 2.25 (1.52) | 2.30 (1.52) | 0.48 | 0.63 |
| Hoarding | 2.04 (1.26) | 2.05 (1.29) | 0.12 | 0.90 |
| Neutralizing | 0.38 (0.72) | 0.40 (0.74) | 0.35 | 0.73 |
| Obsessing | 1.59 (1.40) | 1.56 (1.40) | 0.28 | 0.78 |
| Ordering | 2.23 (1.60) | 2.26 (1.61) | 0.25 | 0.80 |
| Washing | 0.59 (0.82) | 0.64 (0.87) | 0.91 | 0.36 |
| Total | 9.08 (4.08) | 9.21 (4.19) | 0.47 | 0.64 |
| **SDQ scores** | **n=222** | **N=247** | | |
| Emotional Symptoms | 1.88 (1.78) | 1.95 (1.79) | 0.60 | 0.55 |
| Conduct Problems | 1.58(1.54) | 1.74 (1.66) | 1.58 | 0.11 |
| Hyperactivity/Inattention | 3.72 (2.39) | 3.94 (2.48) | 1.40 | 0.16 |
| Peer Relationship Problems | 1.09 (1.38) | 1.21 (1.49) | 1.24 | 0.21 |
| Total Difficulties Score | 8.27 (4.90) | 8.84 (5.31) | 1.74 | 0.08 |
| Prosocial Behavior | 8.69 (1.57) | 8.64 (1.58) | 0.47 | 0.64 |

Note: $^{\wedge}$t and $x^2$ values correspond to one-sample t-tests or $x^2$ tests. ADHD = Attention-Deficit/Hyperactivity Disorder; OCI-CV = Obsessive Compulsive Inventory-Child Version; SDQ = Strengths and Difficulties Questionnaire.

**Table S4: RESULTS OF FUNCTIONAL CONNECTIVITY ANALYSES INCLUDING THE CHILDREN WITH ATTENTION-DEFICIT/HYPERACTIVITY DISORDER SYMPTOMS (N=252)**

| OC Dimension | Brain Region | x, y, z | TFCE | CS |
|---|---|---|---|---|
| Total OC traits | ventral putamen L | -30, 8, -12 | 939.09 | 751 |
| | medial dorsal thalamus L | -12, -16, 14 | 586.77 | 62 |

| OC Dimension | Seed | Brain Region | x, y, z | TFCE | CS |
|---|---|---|---|---|---|
| Obsessing | ventral putamen L | hippocampus R | 24, -22, -6 | 432.56 | 311 |
| | | thalamus L | -22, -20, -4 | 394.19 | 76 |
| | | amygdala L | -22, -8, -12 | 350.95 | 29 |
| Ordering | ventral putamen L | putamen L | -22, 18, -12 | 477.72 | 876 |
| | | rolandic operculum – superior temporal gyrus L | -60, -42, 10 | 465.58 | 374 |
| | | anterior putamen R | 20, 6, 12 | 398.46 | 67 |
| | | posterior putamen R | 30, -14, 6 | 372.65 | 50 |
| | | precentral R | 54, -12, 48 | 359.99 | 33 |
| | | pallidum R | 22, -2, 2 | 351.30 | 18 |
| | | supramarginal L | -52, -28, 30 | 348.45 | 10 |
| Hoarding | ventral putamen L | medial dorsal thalamus L | -8, -18, 8 | 354.48 | 19 |
| | medial dorsal thalamus L | putamen R | 24, 8, 10 | 504.33 | 754 |
| | | cuneus – calcarine | 10, -86, 10 | 442.99 | 1114 |
| | | putamen L | -32, -2, -2 | 424.95 | 1235 |
| | | middle frontal gyrus L | -32, 46, 34 | 253.20 | 290 |
| | | subthalamic nucleus R | 10, -16, -6 | 221.01 | 45 |
| | | medial dorsal thalamus L | -14, -22, 8 | 217.82 | 76 |
| Doubt-Checking | medial dorsal thalamus L | vlPFC – insula L | -46, 30, 20 | 334.81 | 805 |

Note: Anatomic coordinates (x, y, z) are given in Montreal Neurological Institute space. CS = Cluster Size in voxels; L = Left; OC = Obsessive-Compulsive; R = Right; TFCE = Threshold-Free Cluster Enhancement; vlPFC = ventrolateral PreFrontal Cortex.

**Table S5: DEMOGRAPHIC INFORMATION AND OBSESSIVE COMPULSIVE INVENTORY-CHILD VERSION SCORES FOR SEX AND AGE SUBGROUPS**

| **Sample Characteristics** | **Girls (n= 118)** | **Boys (n=109)** | **Statistics** | |
|---|---|---|---|---|
| | *Mean (SD)* | *Mean (SD)* | $t/x^2$ | *p* |
| Age (years) | 9.71 (0.89) | 9.72 (0.83) | 0.08 | 0.94 |
| School Performance (0-5) | 3.88 (0.98) | 3.80 (0.99) | 0.63 | 0.53 |
| Mother's Education (0-5) | 4.49 (0.90) | 4.64 (0.62) | 1.38 | 0.17 |
| **OCI-CV scores** | | | | |
| Doubting-Checking | 2.15 (1.54) | 2.36 (1.49) | 1.02 | 0.31 |
| Hoarding | 2.06 (1.24) | 2.02 (1.28) | 0.24 | 0.81 |
| Neutralizing | 0.33 (0.69) | 0.44 (0.75) | 1.15 | 0.25 |
| Obsessing | 1.56 (1.38) | 1.61 (1.43) | 0.29 | 0.77 |
| Ordering | 2.15 (1.46) | 2.32 (1.73) | 0.79 | 0.43 |
| Washing | 0.61 (0.86) | 0.57 (0.77) | 0.38 | 0.70 |
| Total | 8.86 (3.83) | 9.32 (4.33) | 0.84 | 0.40 |
| **Sample Characteristics** | **Younger (n= 116)** | **Older (n=111)** | **Statistics** | |
| | *Mean (SD)* | *Mean (SD)* | $t/x^2$ | *p* |
| Age (years) | 9.02 (0.45) | 10.44 (0.50) | 22.56 | 0.00 * |
| Sex | | | 0.36 | 0.55 |
| | 62 Girls (53.4%)<br>54 Boys (46.6%) | 56 Girls (50.5%)<br>55 Boys (49.5%) | | |
| School Performance (0-5) | 3.78 (0.98) | 3.90 (0.99) | 0.86 | 0.39 |
| Mother's Education (0-5) | 4.50 (0.86) | 4.62 (0.68) | 1.12 | 0.26 |
| **OCI-CV scores** | | | | |
| Doubting-Checking | 2.29 (1.59) | 2.21 (1.44) | 0.42 | 0.67 |
| Hoarding | 1.97 (1.25) | 2.11 (1.27) | 0.80 | 0.42 |
| Neutralizing | 0.46 (0.78) | 0.31 (0.64) | 1.58 | 0.11 |
| Obsessing | 1.72 (1.45) | 1.44 (1.35) | 1.52 | 0.13 |
| Ordering | 2.31 (1.60) | 2.15 (1.60) | 0.74 | 0.46 |
| Washing | 0.60 (0.82) | 0.58 (0.81) | 0.25 | 0.80 |
| Total | 9.36 (4.33) | 8.79 (3.79) | 1.05 | 0.29 |

Note: OCI-CV = Obsessive Compulsive Inventory-Child Version. *Denotes significant between-group differences ($p<0.05$).

**Table S6: BIVARIATE CORRELATIONS BETWEEN OBSESSIVE COMPULSIVE INVENTORY-CHILD VERSION SUB-SCORES AND AGE**

| | **Doubting-Checking** | **Hoarding** | **Neutralizing** | **Obsessing** | **Ordering** | **Washing** | **Total** |
|---|---|---|---|---|---|---|---|
| **Age** | r=0.04<br>p=0.53 | r=0.09<br>p=0.19 | r=-0.13<br>p=0.06 | r=-0.06<br>p=0.33 | r=-0.07<br>p=0.29 | r=-0.06<br>p=0.39 | r=-0.04<br>p=0.52 |

**Table S7: INDEPENDENT SAMPLE T-TEST OF OBSESSIVE COMPULSIVE INVENTORY-CHILD VERSION SUB-SCORES BETWEEN SEX**

| | Doubting-Checking | Hoarding | Neutralizing | Obsessing | Ordering | Washing | Total |
|---|---|---|---|---|---|---|---|
| **Sex** | t=1.02 | t=0.24 | t=1.15 | t=0.29 | t=0.79 | t=0.38 | t=0.84 |
| | p=0.31 | p=0.81 | p=0.25 | p=0.77 | p=0.43 | p=0.70 | p=0.40 |

## 4.3 Study 3

**Suñol M**, Alemany S, Bustamante M, Diez I, Contreras-Rodríguez O, Laudo B, Macià D, Martínez-Vilavella G, Martínez-Zalacaín I, Menchón JM, Pujol J, Sunyer J, Sepulcre J, Soriano-Mas C. Neurogenetics of attractor dynamic patterns associated with obsessive-compulsive symptoms in healthy children. (In preparation).

# Neurogenetics of attractor dynamic patterns associated with obsessive-compulsive symptoms in healthy children

**Suñol M.[1,2,3,4], Alemany S.[5,6,7], Bustamante M.[5,6,7,8], Diez I.[2,9,10,11], Contreras-Rodríguez O.[1,4], Laudo B.[1], Macià D.[12], Martínez-Vilavella G.[12], Martínez-Zalacaín I.[1,3], Menchón J.M.[1,3,4], Pujol J.[4,12], Sunyer J.[5,6,7,13], Sepulcre J.[2,11]*, Soriano-Mas C.[1,4,14]***

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AUTHORS DECLARE NO CONFLICT OF INTEREST.

**Abstract**

Obsessive-Compulsive Symptoms (OCS) during childhood predispose to Obsessive-Compulsive Disorder (OCD) and are associated with structural and functional brain changes. OCS may arise from disturbed glutamatergic neurotransmission, impairing cognitive fluctuations and promoting over-stable functional states. We aimed at identifying the genetic basis of reverberating connectivity circuits (attractors) associated with OCS in healthy children. 227 schoolchildren (8-12 years) completed the Obsessive-Compulsive Inventory-Child Version and underwent a resting-state fMRI examination. Genome-wide data were obtained from 149 of them. We used a dynamic graph theory-based approach to characterize local and distant attractors and modeled regressions to test associations between OCS and attractors. Next, we compared the spatial similarity of OCS-related attractor maps with a gene expression atlas and assessed overrepresented functional annotations. Finally, we extracted and filtered single-nucleotide polymorphisms (SNPs) modifying gene expression in OCS-related attractor areas and studied whether genetic variants moderated the association between OCS and attractor connectivity. Total OCS negatively correlated with a local attractor in the left ventral putamen and positively with distant attractors in the supplementary motor area and left hippocampus. Genes expressed in the hippocampus contributed to glutamatergic neurotransmission. Ordering OCS positively correlated with a distant attractor in the right superior parietal and genes expressed therein engaged in nervous impulse transmission and cellular transport. SNPs rs7613638 (GRM7), rs11145747 (GNAQ) and rs1994904 (PARVA) moderated the association between total OCS and hippocampus attractor connectivity, whereas rs2143290 (ATP1B1) and rs6490097 (TESC) moderated the relationship between ordering OCS and superior parietal attractor connectivity. Our findings concur with neurobiological OCD models and highlight the potential of multimodal approaches to identify neurobiological signatures of psychiatric symptoms.

## Introduction

Obsessive-Compulsive Disorder (OCD) is characterized by the presence of anxiety-inducing intrusive thoughts (i.e. obsessions) and repetitive behaviors aimed at reducing the increased anxiety levels (i.e. compulsions). OCD is often a chronic condition with a life-time prevalence of 1-3% (Mathes et al., 2019), which imposes significant social and economic burdens (Murray et al., 1996; World Health Organization, 2017). Hence, early characterization of at-risk individuals is fundamental to design prevention strategies that could minimize the impact of OCD. In this vein, evidence suggests that subclinical obsessive-compulsive symptoms (OCS) might herald the onset of OCD in a significant percentage of cases (Black and Gaffney, 2008) and, particularly, that the presence of such symptoms during childhood increases the probability to develop OCD in adulthood (Fullana et al., 2009; Ruscio et al., 2010). Moreover, neuroimaging studies have shown that clinical and subclinical OCS might share common neural underpinnings (Suñol et al., 2018; Suñol et al., 2020).

Substantial amount of evidence from brain functional data, and, mainly, from functional connectivity (FC), indicates that OCS are related to alterations in cortico-striato-thalamic-cortical (CSTC) reverberating loops, which may be driven by an imbalance between the excitatory glutamatergic and the inhibitory GABAergic direct and indirect CSTC circuits (Pittenger, Bloch and Williams, 2011). However, such research typically assumes that functional networks are static over time. Conversely, recent dynamic functional connectivity (DFC) analyses allow a more realistic and detailed approximation to the analysis of CSTC alterations by assessing temporally varying patterns of communication between brain regions (White and Calhoun, 2019). Progress in the study of DFC has allowed to expand the assessment of recurrent dynamic patterns from the neuron-level to the whole-brain scale (Deco et al., 2008), and recent studies have demonstrated that, during resting state, functional streams tend to converge into specific brain nodes. These nodes have been conceptualized as attractors: areas with increased pulling properties that cause DFC to repeatedly converge into them (Diez and Sepulcre, 2018).

Computational neuroscience and, specifically, integrate-and-fire neuronal network simulation models have shown that changes in glutamatergic and GABAergic synaptic efficacy may contribute to specific psychiatric disorders by altering the stability of attractor networks (Rolls, 2012). In the case of OCD, overactivity in glutamatergic excitatory synapses might increase the stability of attractor networks, getting the brain locked in over-stable cognitive states and hindering it to engage in other states, which could lead to obsessive thoughts and subsequent compulsive behavior. This may occur in different brain areas causing distinct symptoms (Rolls et al., 2008; Rolls, 2012), which is congruent with the clinical heterogeneity of OCD and suggests that different OCS might arise from distinct neurobiological alterations (Mataix-Cols and van den Heuvel, 2006; Rolls et al., 2008; Harrison et al., 2013; Nakao et al., 2014). Concurring with these notions, evidence from magnetic resonance spectroscopy (MRS), cerebrospinal fluid (CSF), genetic and treatment studies suggest that glutamatergic dysfunction is central to OCD (Karthik et al., 2020).

MRS and CSF studies have consistently reported increased glutamatergic function in the striatum (Naaijen, et al. 2015) and elevated glutamate concentration in the CSF of individuals with OCD (Chakrabarty et al., 2005; Bhattacharyya et al., 2009). Similarly, genetic studies have provided evidence of significant associations between glutamate related genes (i.e., the SLC1A1 gene) and OCD (Fernandez, Leckman and Pittenger, 2018). Notably, this gene encodes the EAAC-1 glutamate transporter, which ensures low extracellular glutamate concentrations, and alterations in this pathway could therefore lead to abnormally high glutamate levels in the cortex and the striatum (Rotge et al., 2010). Additionally, assessing the interaction between genes associated with OCD has revealed that GRIN2A, GRIN2B and GRIA2 genes, which encode N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunits, have a central role in this disorder, and, overall, glutamate-related pathways are the most impaired in individuals with OCD (Bozorgmehr et al., 2017). Congruently, treatment studies have reported that elevated glutamate levels within the caudate nucleus normalized after treatment with selective serotonin reuptake inhibitors (SSRIs) (Rosenberg et al., 2000), and that glutamate antagonists, such as memantine and riluzole, are useful coadjuvants in OCD treatment (Marinova et al., 2017).

Considering all the above evidence, in the present study we decided to implement a DFC approach to assess the presence of brain nodes with increased attractor properties (i.e. attractors) associated with total and symptom-specific OCS in a large sample of healthy children with subclinical OCS, free from the confounding effects frequently observed in adult OCD samples, such as medication, chronicity and comorbidities. Next, we explored the genetic signature of these attractors by using the Allen Human Brain Atlas (AHBA) and Gene Ontology (GO) enrichment analyses. Finally, we assessed potential genetic risk factors within the Genome-Wide Association (GWA) data of our sample. We hypothesized that expression-modifying single nucleotide polymorphisms (SNPs) in genes relevant to glutamatergic neurotransmission will contribute to impaired glutamate balance and, thus, to the emergence of attractors preventing normal fluctuations between brain states and promoting over-stable brain states, leading to OCS. Additionally, we also hypothesized that specific genetic variants will alter glutamatergic neurotransmission and attractor properties in distinct brain areas, thus leading to specific symptom dimensions. With the combination of multi-source information, we aim to provide an integrated view of the different neurobiological determinants associated to the emergence of obsessive-compulsive symptoms.

## Methods

### Sample Description

The sample of the present study consists of 227 healthy school children, recruited in the framework the BREATHE project (European Commission: FP7-ERC-2010-AdG, ID 268479), which is detailed elsewhere (Sunyer et al., 2015). Briefly, this project assessed the effect of traffic air pollution on neurodevelopment in a sample of 2897 children from 39 schools in Barcelona (Spain). From those, 810 families expressed interest in participating in a neuroimaging sub-study. Four hundred and ninety-one of them were effectively contacted via phone and, finally, 263 children underwent the imaging protocol. Eleven subjects had to be excluded (2 due to a gross structural anomaly, 1 because of an acquisition artifact, 7 due to excessive movement, and 1 because of incomplete psychometric data), resulting in a preliminary sample of 252 children.

To ensure health status, teachers were requested to exclude pupils with special needs and neurological, mental health or other major medical conditions based on the reports of psycho-pedagogical offices. Importantly, teachers were asked not to directly exclude children with attention deficit and hyperactivity disorder (ADHD) symptoms, but instead to evaluate the presence of such symptoms with the ADHD Rating Scale IV, while parents or tutors completed the Strengths and Difficulties Questionnaire (SDQ) (Goodman, 1997). Afterwards, subjects underwent a clinical interview with a psychologist or psychiatrist of the research team who, considering the information provided by teachers and parents or tutors and following the DSM-IV criteria, concluded that none of the participants fulfilled criteria for ADHD or other mental health diagnoses. Nevertheless, 25 children (9.92%) exhibited ADHD symptoms and 6 of them were taking methylphenidate hydrochloride or atomoxetine hydrochloride, hence, we decided to also exclude these subjects, which resulted in the final sample of 227 healthy children. Importantly, this sample was used in previous studies assessing the structural and functional correlates of subclinical OCS (Suñol et al., 2018; Suñol et al., 2020).

Subclinical OCS were self-reported by means of the 21-item Child Version of the Obsessive-Compulsive Inventory (OCI-CV) (Foa et al., 2010), which allows for the assessment of 6 different symptom dimensions: doubt-checking, hoarding, neutralizing, obsessing, ordering and washing, and provides a total symptom score by adding up the subscores of each dimension.

The study was approved by the research ethical committee (number 2010/41221/I) of the IMIM-Parc de Salut Mar, Barcelona, and the FP7-ERC-2010-AdG ethics review committee. Written informed consent was obtained from all parents or tutors.

Imaging Data Acquisition and Preprocessing

Following the recommendations of the FP7-ERC Ethics Review Committee on limiting the exposure to magnetic fields in healthy children, images were acquired using a 1.5 Tesla Signa Excite system (General Electric, Milwaukee, WI, USA) equipped with an 8-channel phased-array head coil and a single-shot echo planar imaging software. Each participant underwent both a structural and a resting-state functional magnetic resonance imaging (rs-fMRI). Anatomical images were obtained using an axial T1-

weighted 3D fast spoiled gradient inversion recovery-prepared sequence. One hundred and thirty-four contiguous slices were acquired with inversion time 400ms; repetition time 11.9ms; echo time 4.2ms; flip angle 15°; field of view 30cm; 256×256-pixel matrix; slice thickness 1.2mm. Structural images were reviewed by a neuroradiologist to detect potential gross anatomical abnormalities. As detailed above, two subjects were excluded because of this reason.

The rs-fMRI acquisition consisted of a gradient recalled acquisition in the steady-state with repetition time 2000ms; echo time 50ms; pulse angle 90°; field of view 24cm; 64×64-pixel matrix; slice thickness 4mm (inter-slice gap1.5mm). Twenty-two interleaved slices were prescribed parallel to the anterior-posterior commissure line covering the entire brain. The rs-fMRI sequence lasted 6 minutes during which participants were instructed to relax, stay awake and lie still while keeping their eyes closed. The first 4 (dummy) images were discarded to allow magnetization to reach equilibrium and 180 whole-brain EPI volumes were acquired for each subject.

MRI data were preprocessed with the CONN Toolbox (Whitfield-Gabrieli and Nieto-Castanon, 2012; Functional Connectivity SPM Toolbox v19: nitrc.org/projects/conn) implemented in MATLAB R2020b (The Math Works Inc, Natick, Mass). The preprocessing of rs-fMRI data followed the default MNI preprocessing pipeline, which consists of realignment and unwrapping, slice-timing correction and ART-based outlier detection (nitrc.org/projects/artifact_detect). Importantly, subjects with less than 4 minutes of non-censored data, according to the ART-based outlier detection step, were excluded on the basis of excessive movement. As detailed above, 7 participants had to be excluded because of this. Next, 3D anatomical images were segmented and normalized to MNI space. This segmentation was later applied to rs-fMRI data, which were then smoothed with an 8mm Gaussian kernel. In a subsequent denoising step, a band-pass filter of 0.008-0.09Hz was applied and realignment parameters, motion outliers of participants with more than 4 minutes of non-censored data (according to the ART-based outlier detection step), a linear detrending term and the white matter and CSF BOLD noise, characterized with the principal component based "aCompCor" method (Whitfield-Gabrieli and Nieto-Castanon, 2012), were regressed out from the time-series. To reduce the computational burden of subsequent analyses, final images were re-

sampled at a voxel size of 6mm. In the course of image conversion and before and after each preprocessing step, all images were inspected by a trained operator to detect acquisition or preprocessing artifacts (1 subject excluded).

Genetic Data Acquisition and Preprocessing

Genetic data were also obtained within the framework of the BREATHE project. Genotyping procedure is detailed in (Alemany et al., 2017). In brief, DNA samples were collected from saliva with the Oragene DNA OG-500kit (DNA Genotek) and were quantified through Quant-iTTM PicoGreen® dsDNA Assay Kit (Life Technologies). Genome-wide genotyping was performed in 1778 children using the HumanCore BeadChip WG-330-1101 (Illumina) at the Spanish National Genotyping Centre (CEGEN). PLINK V1.9 (Purcell et al., 2007; cog-genomics.org/plink/1.9) was used to ensure genotyping quality and 111 subjects were excluded during this process, leaving 1667 individuals. Genetic variants were filtered by Hardy-Weinberg equilibrium ($p<10^{-6}$), allele frequency, excluding minor allele frequency (MAF<1%), and a SNP call rate with a minimum of 95%. Polymorphisms were imputed using IMPUTE2 v2 (Howie et al., 2009), taking the 1000 Genomes project phase I integrated variant set (www.1000genomes.org) as a reference haplotype panel. From the genotyped sample, 149 individuals had good-quality MRI data and met the inclusion criteria. Hence, the genetic analyses of our study were performed in this sample of 149 healthy children.

Data analyses

*Sociodemographic and Psychometric Data*

Statistical analyses of sociodemographic and psychometric data were performed with SPSS v23 (IBM Corp, Armonk, NY).

*OCS-related Attractor Connectivity Analyses*

We implemented a graph theory-based method named Stepwise functional connectivity (SFC) that characterizes DFC changes over time (Sepulcre et al., 2010; Sepulcre et al., 2012; Diez and Sepulcre, 2018). The version of the method we used is detailed in Diez and Sepulcre (2018). It allows to detect, at the whole-brain level, brain nodes in which DFC streams converge repeatedly across time, proxy of attractors (see Figure 1).

First, we used a whole-brain grey matter mask of 6185 voxels (voxel size: 6mm) to extract BOLD time-series and we applied a sliding window approach. rs-fMRI data were split into time windows of 30s each (TR=2s; 15 time points), with 1 lagged time point between them to achieve smooth transitions (Tognoli and Kelso, 2014). Then, we computed Pearson's correlations between the BOLD time-series of all the voxels in each time window to generate a 6185x6185 association connectivity matrix for each time window (see Figure 1A). Negative and non-significant positive correlations (p-values>0.05) were removed to eliminate connections likely attributable to noise (Van Dijk et al., 2010). Next, we performed a variance-stabilizing Fisher's transformation to all correlation coefficients in the matrices before performing further analyses.

Since high connectivity strength is observed between neighbor nodes, assessing total connectivity would mainly reflect local connectivity while overshadowing distant patterns. Hence, we computed dynamic whole-brain SFC for each functional matrix of each time window under two separate conditions considering: (1) exclusively direct neighbor connections through triangle motifs (i.e. local or modular connectivity), and (2) connections outside the local neighborhood (i.e. distant connectivity) (see Figure 1B). Importantly, since the brain network was segregated in triangle motifs to assess connectivity inside and outside these modules, the terms local and distant refer to network-based topology and not Euclidean distances within the human brain (Diez and Sepulcre, 2018). The SFC method consisted of matrix multiplications using Matlab R2020b (The Math Works Inc, Natick, Mass) and was performed simultaneously for all voxels in the mask under the two conditions (i.e. local and distant SFC). Within each discrete sliding window, the corresponding association matrix was used to calculate the connectivity steps from each node to the rest of the nodes in the graph topological space until seven steps were completed. The seven-step distance was selected based on previous research on path lengths between nodes in FC graphs (Sepulcre et al., 2012), which suggests that more than seven steps do not improve the communicability between nodes. Finally, we computed the mean of all SFC weighted degree maps containing the connectivity convergences from each time window to obtain a single local and distant SFC map for each subject (see Figure 1C). In these final maps, increased dynamic SFC degree values in a given voxel indicate higher connectivity streams

repeatedly converging at that specific voxel, proxy of attractor, while smaller SFC degree show lower DFC convergences (see Figure 1D).

In order to test associations between OCS and local and distant attractors, we used the Statistical Parametric Mapping 12 software (SPM12; The Wellcome Department of Imaging Neuroscience, London, UK) to model four multiple regression models exploring the associations between total OCI-CV scores and local (i.e., model 1) and distant (i.e., model 2) SFC maps and between symptom-specific OCI-CV scores and local (i.e., model 3) and distant (i.e., model 4) SFC maps. In the symptom-specific models, doubt-checking, hoarding, obsessing and ordering OCI-CV scores were added as predictor variables, whereas neutralizing and washing OCI-CV scores were only included as nuisance covariates because of lack of variance (mean values below 1 and median values of 0). Additionally, in all models, age, sex and degrees of freedom from first-level analyses were included as nuisance variables. This latter variable was added to the models to compensate for different across-subject data acquisition points caused by the outlier volumes regression during the denoising preprocessing step. Importantly, results were independently evaluated within a cortical and a subcortical mask to maximize analysis sensitivity in these two brain compartments. Statistical significance was set at $p<0.05$, family-wise error (FWE) corrected at the cluster-level.

*Genetic Signature of OCS-Related Attractors*

To better understand the neurobiological underpinnings of our findings, we assessed the association between local and distant OCS-related attractor maps and the microarray gene expression data from the Allen Human Brain Atlas database, which provides whole-brain, high-resolution genome-wide expression values for six human subjects, quantifying more than 20,000 genes in 3702 spatially distinct brain samples (Hawrylycz et al. 2012; http://human.brain-map.org).

Consistent with the recommendations proposed in a previous study (Arnatkevic et al., 2019), we firstly implemented an anatomical transformation of the transcriptional profiles of 20,787 protein-coding genes based on 68 cortical regions defined by the Desikan atlas (Desikan et al., 2006; French and Paus, 2015) and 16 subcortical regions from the Freesurfer segmentation (Fischl et al., 2002; Fischl et al., 2004). Secondly, we

corregistered the OCS-related local and distant attractor maps from the voxel-level to the 68 cortical and the 16 subcortical regions. Thirdly, we masked these maps with either a cortical or a subcortical mask, depending on the regions where significant OCS-related attractors were located, and we used Pearson's correlations to assess the spatial similarity value of each cortical or subcortical gene's expression with the masked local and distant OCS-related attractor maps, separately. Fourthly, we identified genes with a value higher than 2 standard deviations above the mean of the spatial similarity distribution. This method allowed to detect cortical and subcortical genes the expression of which was spatially similar to the local and distant OCS-related attractor maps, respectively (See Figure 1E).

Next, we performed a Gene Ontology (GO) Protein Analysis Through Evolutionary Relationships (PANTHER) enrichment analyses (Ashburner, et al. 2000; Gene Ontology Consortium, 2015; Mi et al., 2019; PANTHER 14.1 software implemented in geneontology.org) to elucidate whether genes associated with OCS-related attractor maps had overrepresented functional annotations. We used the GO Biological Processes annotation dataset, which, according to geneontology.org, is centered on biological programs accomplished by multiple molecular activities. Significant results were determined by a Fisher's Exact test with a false discovery rate (FDR)-corrected significance threshold of $p<0.05$ FDR and a fold enrichment>2.

The SNPs of the genes involved in overrepresented functions that modified gene expression in OCS-related attractor areas were located using the single-tissue expression quantitative trait loci (eQTLs) viewer implemented in the Genotype-Tissue Expression (GTEx) Project Portal (GTEx Consortium, 2013; GTEx Portal V8: gtexportal.org). The obtained SNPs underwent a linkage disequilibrium filtering (LDF) using the CEU population group and cutoffs of $R^2=0.1$ and MAF=0.05 to ensure equilibrium (Machiela and Chanock, 2015; 2017; ldlink.nci.nih.gov/?tab=snpclip).

The final SNPs doses were extracted from our GWA data with PLINK V1.9 (Purcell et al., 2007; cog-genomics.org/plink/1.9). For each SNP, the sample was split into two subgroups according to the presence of minor alleles (0 versus 1 or 2 copies of the minor allele). Then, we extracted the eigenvalues of the connectivity peaks from the OCS-

related attractor maps and evaluated correlations between these values and the OCI-CV scores within each SNP subgroup with SPSS v23 (IBM Corp, Armonk, NY), effectively assessing moderating effects of gene expression on the correlations between imaging and psychometric data. These correlations were statistically corrected with a Bonferroni procedure and contrasted using a Fisher r-to-z transformation.

## Results

### Sociodemographic and Psychometric Data

Sociodemographic and psychometric variables for the whole sample of participants are displayed in Table 1. OCI-CV and SDQ scores were similar and, in some cases, lower than the ones obtained in normative pediatric samples (see Tables S1 and S2). Interviews with psychiatrists or psychologists revealed no cases likely to receive a mental health diagnosis.

### Attractor Connectivity and Obsessive-Compulsive Symptoms

At the subcortical level, we found a negative association between total OCI-CV score and a local attractor located in the left ventral putamen (see Figure 2 and Table 2), and a positive correlation between total OCI-CV score and a distant attractor in the left hippocampus (see Figure 2 and Table 2). At the cortical level, we observed a positive association between total OCI-CV score and a distant attractor encompassing the bilateral supplementary motor area (SMA) (see Figure 2 and Table 2). Finally, in symptom-specific analyses, we observed a positive correlation between the ordering OCI-CV subscore and a distant attractor located at the right superior parietal cortex (see Figure 2 and Table 2). No further correlations were detected with the rest of symptom-specific scores.

### Genetic Signature of Obsessive-Compulsive Symptom-Related Attractors

To better understand the neurobiological basis of the associations between the attractors and subclinical OCS, we compared our T-maps with the cortical and subcortical gene expression (20,787 genes) from the Allen Human Brain Atlas.

At the subcortical level, we found that the total symptom-related local attractor map, encompassing the left ventral putamen, co-located with the expression levels of 44 subcortical genes (see Table S3). However, Gene Ontology (GO) enrichment analyses revealed no significant functional overrepresentations for these genes. In contrast, the total symptom-related distant attractor map encompassing the left hippocampus was similarly distributed as the expression levels of 605 subcortical genes (see Table S3), of which, according to GO enrichment analysis, 289 genes were significantly engaged in 124 biological processes, including glutamatergic synaptic transmission, glutamate receptor signaling pathway, synaptic processes, learning, and fear and defense responses, among many others (see Table S4). Using the GTEx Portal and limiting our SNPs search to the GTEx tissue label 'brain - hippocampus', we detected that 47 of the 289 subcortical genes had SNPs that modulated their expression in the hippocampus, where we located the total symptom-related distant attractor. Then, we extracted the SNPs of these 47 genes, and we obtained a preliminary list of 1151 SNPs that, after LDF, was reduced to 23 SNPs. Twenty-two of these 23 SNPs were imputed in the GWA data of our sample; hence, we retrieved the doses of these final 22 SNPs from the GWA data and evaluated whether genetic variants moderated the association between total symptoms and connectivity of the left hippocampus.

We observed that the positive association between the total OCI-CV score and distant attractor connectivity of the left hippocampus was only significant in the groups with 1 or 2 copies of the minor alleles C in the SNPs rs7613638 of the GRM7 gene (0 copies, r=0.069; 1 or 2 copies, r=0.436; Z=2.18; p=0.029), rs11145747 of the GNAQ gene (0 copies, r=0.153; 1 or 2 copies, r=0.457; Z=1.99; p=0.046) and rs1994904 of the PARVA gene (0 copies, r=0.184; 1 or 2 copies, r=0.724; Z=3.54; p=0.0004) (see Figure S1). Notably, GRM7 and GNAQ are genes involved in glutamatergic neurotransmission, whereas PARVA contributes to actin cytoskeleton regulation and, therefore, to cell adhesion, motility and survival.

At the cortical level, we observed that the total symptom-related distant attractor map, comprising the SMA, co-located with the expression levels of 210 cortical genes (see Table S3). Nevertheless, GO enrichment analysis showed that these genes did not engage in overrepresented functions. Conversely, the ordering symptom-related distant

attractor map, encompassing the right superior parietal cortex, was similarly distributed as the expression levels of 413 cortical genes (see Table S3) and, based on GO enrichment analysis, 101 of them significantly contributed in 25 biological processes, including transmission of nerve impulse and cellular transport (see Table S5). Using the GTEx Portal, we limited the SNPs retrieval of these genes to the GTEx tissue label 'brain - cortex' and found that 32 of the 101 cortical genes had expression-modifying SNPs in this area. After retrieving the SNPs of these 32 genes, we had a list of 2052 SNPs that, after LDF, was reduced to 49 SNPs. 36 of these 49 SNPs were imputed in the GWA data of our sample. Therefore, we extracted the doses of these final 36 SNPs from the GWA data and assessed whether genetic variants moderated the correlation between ordering symptoms and connectivity of the right superior parietal cortex.

We observed that the positive correlation between ordering OCI-CV subscores and distant attractor connectivity of the right superior parietal cortex was only significant in the group with 1 or 2 copies of the minor allele T in the SNP rs2143290 of the ATP1B1 gene (0 copies, r=0.134; 1 or 2 copies, r=0.647; Z=3.12; p=0.002), and in the group with 0 copies of the minor allele C in the SNP rs6490097 of the gene TESC gene (0 copies, r=0.406; 1 or 2 copies, r=-0.008; Z=2.14; p=0.032) (Figure S2). The ATP1B1 gene is contributes to the establishment and maintenance of electrochemical gradients across the plasma membrane, whereas the TESC gene is involved in controlling cellular pH.

**Discussion**

To the best of our knowledge, this study is the first attempt to combine multi-source neurobiological information to assess the relationship between total and symptom-specific subclinical OCS with dynamic attractor connectivity and its putative genetic signature. Remarkably, our findings indicated that total OCS correlated with attractor connectivity in nodes of the CSTC circuits, such as the ventral putamen and the SMA, which is congruent with prevailing neurobiological models of OCD (Menzies et al., 2008; van den Heuvel et al., 2016), and adds to the evidence that these circuits are already altered at subclinical or preclinical stages, irrespective of medication, comorbidities or other confounding factors characteristic of clinical OCD samples. Nevertheless, we also

found that total and ordering subclinical OCS were linked to attractor connectivity in regions outside the traditional boundaries of the CSTC loops, such as the hippocampus and the superior parietal cortex. Nevertheless, these findings concur with evidence suggesting the involvement of areas outside these circuits in the development of OCS (Chamberlain et al., 2008; Menzies et al., 2008; Milad and Rauch, 2012), and also support the notion that, albeit some functional alterations may be common to distinct symptom dimensions, some brain functional features may be specific to certain symptom dimensions (Harrison et al., 2013; Mataix-Cols et al., 2004; Mataix-Cols and van den Heuvel, 2006). Importantly, combining imaging transcriptomics and the GWA data of our sample, we were able to identify genetic variants that modified gene expression in symptom-related attractor areas, which significantly moderated the association between symptoms and attractor connectivity. Hence, we were able to put forward a preliminary set of genetic variants that act as risk modulators for altered attractor connectivity and, therefore, for the development of OCS.

Focusing on the neurobiological correlates of total OCS, at the cortical level we observed a positive correlation between these symptoms and a distant attractor cluster located at the SMA. This area is involved in planning and preparation of future performance, as well as in the initiation, inhibition, maintenance, and repetition of action (Nachev, Kennard and Husian, 2008). Moreover, as a part of the sensorimotor CSTC loop, the SMA contributes to mediate automatic responses and the transition from goal-directed to habitual behaviors (van den Heuvel et al., 2016). In the framework of the sensorimotor CSTC circuit, the putamen receives inputs from motor and sensory cortical regions in a somatotopic manner. From there, topographically specific projections reach the motor sections of the outer globus pallidus (GPe), the inner globus pallidus (GPi) and substantia nigra pars reticulata (SNr). In turn, projections from motor portions of the GPi and SNr are directed to the motor thalamus (ventral lateral and ventral anterior nuclei), from where information is projected to the SMA and other motor and premotor cortical areas (DeLong and Wichmann, 2010). Hence, our finding may be reflecting increased subcortical outputs converging and reverberating in the SMA of individuals with OCS. This may alter connectivity within the sensorimotor CSTC loop, which may result in an impaired ability to disengage from ongoing overt behaviors and a stereotyped pattern

of motor response. This interpretation is congruent with previous meta-analyses highlighting the efficacy of low-frequency repetitive transcranial magnetic stimulation (LF-rTMS) targeting the SMA on improving OCS (Berlim, Neufeld and Van den Eynde, 2013; Zhou et al., 2017). Such improvement could be partially accounted for by the inhibitory effects of LF-rTMS on normalizing hyperconnectivity of the SMA and, thus, on normalizing sensorimotor CSTC loop activity in individuals with OCD. Nevertheless, future research is warranted to determine whether the distant attractor located at the SMA is also associated with OCS in clinical samples.

At subcortical level, we found that total OCS negatively correlated with local attractor properties of the left ventral putamen. From a functional perspective, the ventral putamen is the main subcortical relay station of the ventral cognitive CSTC circuit, which contributes to successful response inhibition and cognitive control of emotion (van den Heuvel et al, 2016). Hence, our finding of a negative symptom-related local attractor in the ventral putamen can be interpreted as this region becoming more functionally segregated, dispersing connectivity inputs from neighboring nodes, which could then alter functioning in the whole ventral cognitive CSTC loop. At the behavioral level, such interpretation concurs with evidence showing that functions mediated by this loop are impaired in subjects with OCD and, in some cases, also in their first-degree relatives (Chamberlain et al., 2008; Menzies et al., 2007; 2008; Lennertz et al., 2012; Milad and Rauch, 2012). Moreover, previous studies have reported reduced functional connectivity within the ventral cognitive loop both in youth and adults with OCD (Harrison et al., 2009; Bernstein et al., 2016).

Importantly, the negative association between total OCS and left ventral putamen connectivity was also observed in a previous study with this same sample of children that assessed static functional connectivity not segregating into local and distant connections (Suñol et al., 2020). Nevertheless, with the present dynamic approach this finding was limited to local connections, suggesting that neighboring nodes exhibit higher connectivity strength that more distant ones. In consequence, total connectivity assessments tend to emphasize local connectivity, partially watering down information from more distant areas (Diez and Sepulcre, 2018). In any case, our finding also concurs with a previous study describing a negative association between total resting-state

activity of the ventral putamen and the Y-BOCS compulsions subscore in adults with OCD (Giménez et al., 2017). Conversely, the only study that assessed local and distant resting-state functional connectivity alterations in clinical OCD (Beucke et al., 2013) reported that local connectivity in the left ventral putamen was specifically reduced in medicated subjects, whereas increased distant connectivity in this region correlated with OCD symptom severity. Nevertheless, the different results between Beucke et al (2013) and the present study could indeed reflect differences between subclinical and clinical OCS or between youth and adults with OCS. Likewise, they may also reflect methodological differences between the studies, specifically, in the use of a static vs. a dynamic functional connectivity assessment and a segregation approach based on Euclidean distances rather than on topological network properties.

At the subcortical level, we observed a positive association between total OCS and the distant attractor properties of the left hippocampus. From a functional perspective, the hippocampus is an important brain hub that receives multiple cortical inputs through the entorhinal cortex and is involved in mnemonic processing, spatial cognition, and approach-avoidance conflict, modulating behavior when individuals are both attracted to and repelled by the same goal (Battaglia et al., 2011; Ito and Lee, 2016). The increased attractor properties of the hippocampus observed in this study may cause dynamic connectivity from distant cortical areas (e.g., association and prefrontal cortices) to reach the hippocampus through the glutamatergic fibers of the perforant path and converge and reverberate within this region, hampering its multiple functions. This interpretation concurs with previous evidence suggesting that individuals with OCD show positive memory biases favoring threat-related stimuli (Muller and Roberts, 2005), as well as a biased avoidance tendency (Endrass et al., 2011). Our finding is also in agreement with preliminary evidence showing that increased glucose metabolism in the hippocampus correlated with OCS in individuals with OCD (Kwon et al., 2003), and with a recent meta-analysis reporting increased GM volumes in the hippocampus irrespective of medication status (Picó-Pérez et al., 2020). However, decreased hippocampal volumes have also been reported in adults with OCD, especially in individuals taking medication, with later OCD onset, or with comorbid depression (Boedhoe et al., 2017).

Notably, in agreement with the OCD attractor hypothesis of Rolls et al. (2008), we were also able to link the increased attractor properties of the hippocampus to glutamatergic neurotransmission. Firstly, we observed that the genes significantly expressed in this area were functionally engaged in biological processes related to glutamatergic neurotransmission and, secondly, we found that, in our sample, SNPs of glutamate-related genes such as GRM7 and GNAQ acted as risk factors for symptom-related attractor connectivity in this area. GRM7 encodes the G protein-coupled metabotropic glutamate receptor 7 (mGluR7) which, as a member of the Group III metabotropic glutamate receptors, decreases NMDA receptor activity at the presynaptic level (Fisher et al., 2018) and modulates the strength and timing of excitatory transmission in the hippocampus (Cosgrove et al., 2011). In humans, polymorphisms in the GRM7 have been associated with anxiety and mood disorders, schizophrenia, autism and ADHD (Alliey-Rodriguez et al., 2011; Saccetti et al., 2017; Fisher et al., 2018; Noroozi et al., 2019), suggesting that glutamatergic neurotransmission may be impaired in multiple psychiatric conditions. Moreover, mGluR7-knockdown in the hippocampus and basolateral amygdala of mice caused deficits in the extinction of learned aversion, whereas mGluR7 activation reduced the acquisition of conditioned fear and facilitated its extinction (Fendt et al., 2008). In this context, our finding may tentatively indicate that polymorphisms that reduce or impair GRM7 expression could lead to increased glutamatergic neurotransmission and deficits in fear extinction learning. In contrast, GNAQ encodes Gαq, which activates intracellular signaling pathways in response to activation of cell surface Gαq-protein-coupled receptors (GPCRs). mGluR1, mGluR5 are GPCRs, and, together with other GPCRs, regulate NMDA receptor activity in the hippocampus (MacDonald, Jackson and Beazely, 2007). In humans, altered GNAQ expression has been reported in individuals with schizophrenia (MacDonald et al., 2015), and, in animal models, a previous study showed that Gαq-deficient mice lacked metabotropic glutamate receptor-dependent long-term depression while maintaining normal long-term potentiation in the hippocampus (Kleppisch et al., 2001). Moreover, conditional knockout mice, in whom Gαq was deleted from glutamatergic cells within the hippocampus, the frontal cortex and the amygdala, exhibited spatial memory deficits and enhanced behavioral responses to psychostimulants, albeit no changes in anxiety responses (Graham et al., 2015). Hence, polymorphisms that alter GNAQ

expression in the hippocampus may contribute to alter glutamate-dependent synaptic transmission and plasticity in the hippocampus and, in consequence, increase distant attractor properties of this region, leading to the development of OCS.

Additionally, we found that a SNP of the PARVA gene also acted as a risk factor of symptom-related attractor connectivity in the hippocampus. This gene encodes the α-parvin, an actin-binding protein that is part of the integrin-linked kinase signaling complex and contributes to cell adhesion, motility and survival. This gene is nearly ubiquitously expressed and, although it has not been directly associated to OCD, a study showed that regulation of actin cytoskeleton, cell adhesion molecules and actin binding may be pathways associated with an increased risk for OCD (Yue et al., 2016). Hence, when alterations in PARVA expression are observed in the hippocampus, they could result in altered hippocampal connectivity patterns and the resulting emergence of OCS linked to these altered pathways.

Regarding symptom-specific analyses, we found a positive correlation between ordering symptoms and a distant attractor located at the right superior parietal cortex. At the functional level, the superior parietal cortex is involved in visuospatial and attentional processing, including the representation and manipulation of objects, as well as in executive functions (Johns, 2014). Moreover, computational and empirical studies have suggested that this region is a multimodal brain hub that contributes to the integration of information from distributed cortical regions (van den Heuvel and Sporns, 2011; 2013). Following the hypothesis of Rolls and Deco (2008), noting that distinct obsessive-compulsive symptoms may arise from increased attractor properties in different brain areas, we interpreted that increased distant attractor properties in the superior parietal cortex may cause dynamic connectivity from distant cortical regions to converge and reverberate in this area. This could lead to the brain getting locked in specific overstable states that impair the adaptive and flexible functioning of visuospatial and attentional processing and executive functions, which could predispose to ordering symptoms. This interpretation is in line with a recent meta-analysis, reporting that the symmetry/ordering symptoms were more strongly related to poorer neuropsychological performance in attention and visuospatial tasks than obsessive/checking symptoms (Bragdon, Gibb and Coles, 2018). Likewise, our results also concur with a previous study

in which the ordering/symmetry dimension correlated with structural abnormalities in the parietal cortex (van den Heuvel et al., 2009).

Importantly, we found that a polymorphism of the ATP1B1 gene acted as a risk factor for the ordering symptom-related right superior parietal attractor, whereas a polymorphism of the TESC gene acted as a protective factor. Although these genes have not been previously associated with ordering symptoms or OCD, they have been reported to be differentially expressed in bipolar disorder and schizophrenia when compared to healthy controls (Smolin et al., 2012; Logotheti et al., 2013). ATP1B1 encodes the β1 subunit of the Na+/K+ ATPase (NKA), which is an integral membrane protein responsible for establishing and maintaining the electrochemical gradients of sodium and potassium ions across the plasma membrane. These gradients are vital to sodium-coupled transport and to electrical excitability and, therefore, to synaptic transmission. For instance, the transmembrane Na+ gradient generated by the NKA is crucial for astrocytic uptake of glutamate, released after activation of excitatory synapses (Illarionova et al., 2014). Moreover, there is preliminary evidence of crosstalk between the NKA and the NMDA receptors (de Lores Arnaiz and Bersier, 2014). Notably, within the NKA, the β subunit regulates, through assembly of α/β heterodimers, the number of sodium pumps transported to the plasma membrane. Hence, polymorphisms of the ATP1B1 gene, modifying its expression at the superior parietal cortex, could contribute to alter the excitability of these neurons, increasing the attractor properties of this area and, consequently, predisposing to ordering symptoms.

On the other hand, TESC encodes for Tescalcin, a calcium-binding protein that regulates cellular pH by controlling the NA+/H+ exchanger 1 (NHE1) activity at the plasma membrane. It promotes maturation, transport, cell surface stability and exchange activity of NHE1 at the plasma membrane, which contributes to neurite morphogenesis (Sin et al., 2009). Notably, reduced activity of the NHE1 prevents NMDA receptors-induced excitotoxicity (Lam et al., 2012). We hypothesize that polymorphisms of TESC could modify its expression in a manner that favors NHE1 regulation and prevents increases in glutamatergic neurotransmission and, in consequence, attractor properties in the superior parietal cortex.

This study is not without limitations. Although recruiting healthy children as research participants has the advantage of avoiding the effects of traditional confounders of clinical OCD samples, such as medication status, presence of different comorbidities or the clinical and neurobiological features associated with disorder's course, our study lacked of an age- and sex-matched group of participants diagnosed with OCD. Hence, we were not able to determine whether our neurobiological findings are also present in OCD samples. Moreover, it should be noted that longitudinal studies are warranted to ascertain whether our findings are indeed predictive of OCD development or solely linked to subclinical OCS. Also, we did not fully control for other potential confounders, such as the presence of psychiatric or neurological conditions in first-degree relatives, and participants underwent a single clinical interview, which prevented obtaining intra- or inter-rater reliability measures validating study inclusion. Finally, due to the lack of previous studies implementing dynamic attractor connectivity approaches and combining multi-source neurobiological information to assess total and dimensional OCS, the interpretations of our imaging and genetic findings may be perhaps somewhat speculative and should be considered with caution.

In conclusion, our results add further evidence to the link between OCS and CSTC circuit alterations, even at subclinical stages, while also highlighting the important role of areas outside these circuits. Furthermore, our findings support the hypotheses put forward by earlier integrate-and-fire neuronal network simulation studies suggesting that OCS may arise as a consequence of altered attractor properties in certain brain nodes caused by overactive glutamatergic synapses and, more specifically, that distinct OCS may be associated with altered attractor properties occurring in different brain areas (Rolls et al., 2008). In any case, beyond the glutamatergic neurotransmission-based attractor hypothesis, our findings also suggest that alterations in other biological processes in neurons of specific brain areas, such as the maintenance of cellular electrochemical gradients and pH or the regulation of actin cytoskeleton, could be involved in the formation of OCS-related attractors. Further research is warranted to characterize the link between alterations in these biological processes and the emergence of attractors. Nevertheless, it is also important to note that we were able to identify not only the genetic signature and biological processes linked to symptom-related attractors, but

also the specific genetic variants that, in our sample, acted as risk or protective factors in relation to the presence of such symptom-related attractors. Taken together, such imaging and genetic features may be seen as putative biomarkers of increased risk for developing a full-blown disorder, also accounting for the clinical heterogeneity of OCD.

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**Conflict of Interest:**

Drs. Alemany, Bustamante, Diez, Contreras-Rodríguez, Macià, Menchón, Pujol, Sunyer, Sepulcre, Soriano-Mas, Mss. Suñol, Laudo, Messrs. Martínez-Vilavella, and Martínez-Zalacaín report no biomedical financial interests or potential conflicts of interest.

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**Table 1.** Sociodemographic and psychometric data

| **Sample characteristics (n= 227)** | | |
|---|---|---|
| | *Mean (SD)* | *Median* |
| Age (years) | 9.71 (0.86)<br>Range: 8-12.1 | 9.67 |
| Sex | | |
| Girls, n(%) | 118 (52%) | |
| Boys, n(%) | 109 (48%) | |
| Non-Psychiatric Medication, n(%) | 19 (8.4%)[a] | |
| School Performance (0-5) | 3.84 (0.98) | 4 |
| Mother's Education (0-5) | 4.56 (0.78) | 5 |
| **OCI-CV scores (n= 227)** | | |
| | *Mean (SD)* | *Median* |
| Doubt-Checking | 2.25 (1.52) | 2 |
| Hoarding | 2.04 (1.26) | 2 |
| Neutralizing | 0.38 (0.72) | 0 |
| Obsessing | 1.59 (1.40) | 1 |
| Ordering | 2.23 (1.60) | 2 |
| Washing | 0.59 (0.82) | 0 |
| Total | 9.08 (4.08) | 9 |

[a] Non-Psychiatric medication included acetaminophen, antihistamines or antibiotics.

OCI-CV: Obsessive Compulsive Inventory-Child Version; SD: Standard Deviation.

**Table 2.** Associations between Local/Distant Attractor Connectivity and Obsessive-Compulsive Symptoms.

| Mask | Attractor | Contrast | Brain Region | x, y, z | T | p-FWE | CS |
|---|---|---|---|---|---|---|---|
| ***Total OCI-CV*** | | | | | | | |
| Subcortical | Local | Negative | Ventral Putamen L | -22, 16, -3 | 3.81 | 0.007 | 11 |
| | Distant | Positive | Hippocampus L | -28, -9, -21 | 4.01 | 0.033 | 6 |
| Cortical | Distant | Positive | SMA R/L | 3, -15, 58 | 3.9 | 0.042 | 11 |
| ***Ordering OCI-CV*** | | | | | | | |
| Cortical | Distant | Positive | Sup Parietal R | 15, -57, 58 | 4.13 | 0.031 | 12 |

Anatomic coordinates (x, y, z) are given in Montreal Neurological Institute space. The voxel size is 6x6x6mm; CS: cluster size in voxels; L: left; OCI-CV: Obsessive Compulsive Inventory-Child Version; p-FWE: p-value Family-Wise Error corrected at cluster-level; SMA: Supplementary Motor Area; Sup: Superior; R: right.

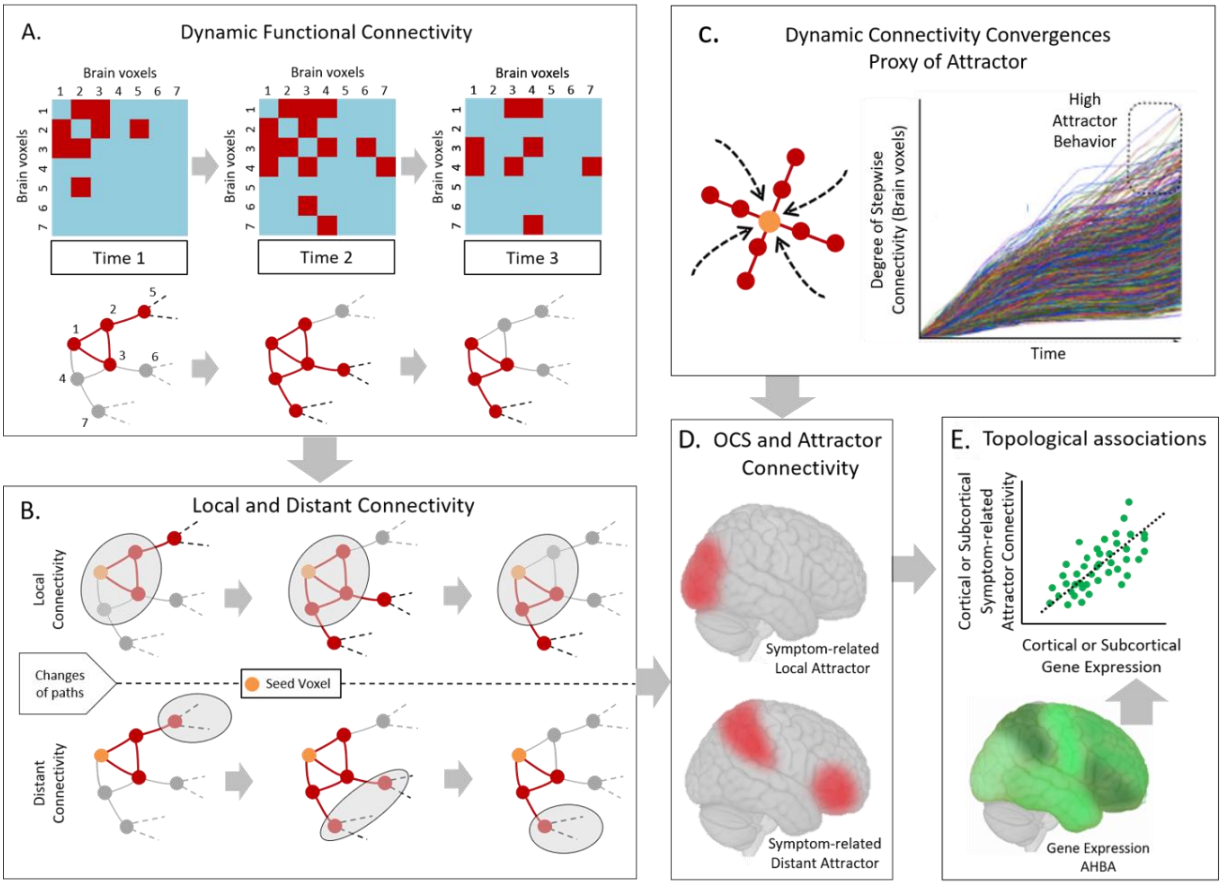


**Figure 1. A.** Dynamic functional connectivity was assessed through association matrices over time windows that represent distinct network configurations over time. Top: illustrative representation of three association matrices at three time points. Bottom: illustrative representation of network configurations at three time points. **B.** Total connectivity was segregated into local (or modular) and distant connectivity via triangle motifs. Top: illustrative representation of local connectivity at three time points. Bottom: Top: illustrative representation of distant connectivity at three time points. **C.** Stepwise functional connectivity analyses were implemented to assess dynamic functional streams convergence at specific brain voxels. Left: illustrative representation of streams converging at a specific brain voxel, proxy of attractor. Right: whole-brain calculation of recurrence of streams converging at voxels across time (adapted from Diez and Sepulcre, 2018). **D.** Connectivity segregation allowed to assess local and distant connectivity convergences targeting specific areas (i.e. attractors) and their relationship with OCS. For illustration purposes, schemes represent hypothetical brain regions with high local (top) or distant (bottom) connectivity convergences associated with symptoms. **E.** Local and distant symptom-related attractor connectivity maps were spatially compared with the transcriptome of the AHBA, separately. Top: theoretical scatterplot of the spatial similarity regression. Bottom: illustrative representation of the whole-brain gene expression AHBA. AHBA: Allen Human Brain Atlas; OCS: Obsessive-Compulsive Symptoms. This figure was adapted from Diez and Sepulcre (2018).

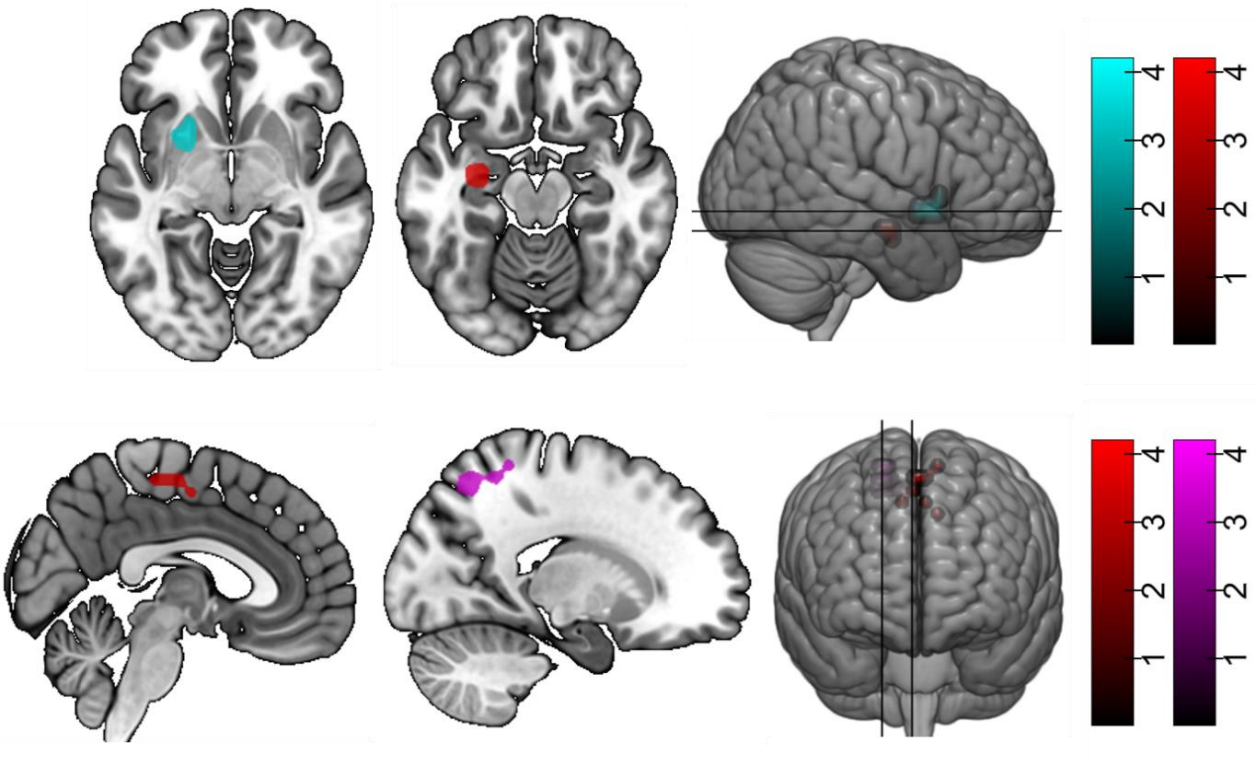


**Figure 2.** Associations between Local/Distant Attractor Connectivity and Obsessive-Compulsive Symptoms. Cyan color represents reduced local attractor connectivity associated with total Obsessive-Compulsive Inventory-Child Version (OCI-CV) score. Red color represents increased distant attractor connectivity associated with total OCI-CV score. Violet color represents increased distant attractor connectivity associated with ordering OCI-CV score. Color bars indicate T values. Images are displayed in neurological convention.

Supplementary Material:

# Neurogenetics of attractor dynamic patterns associated with obsessive-compulsive symptoms in healthy children

**INDEX:**

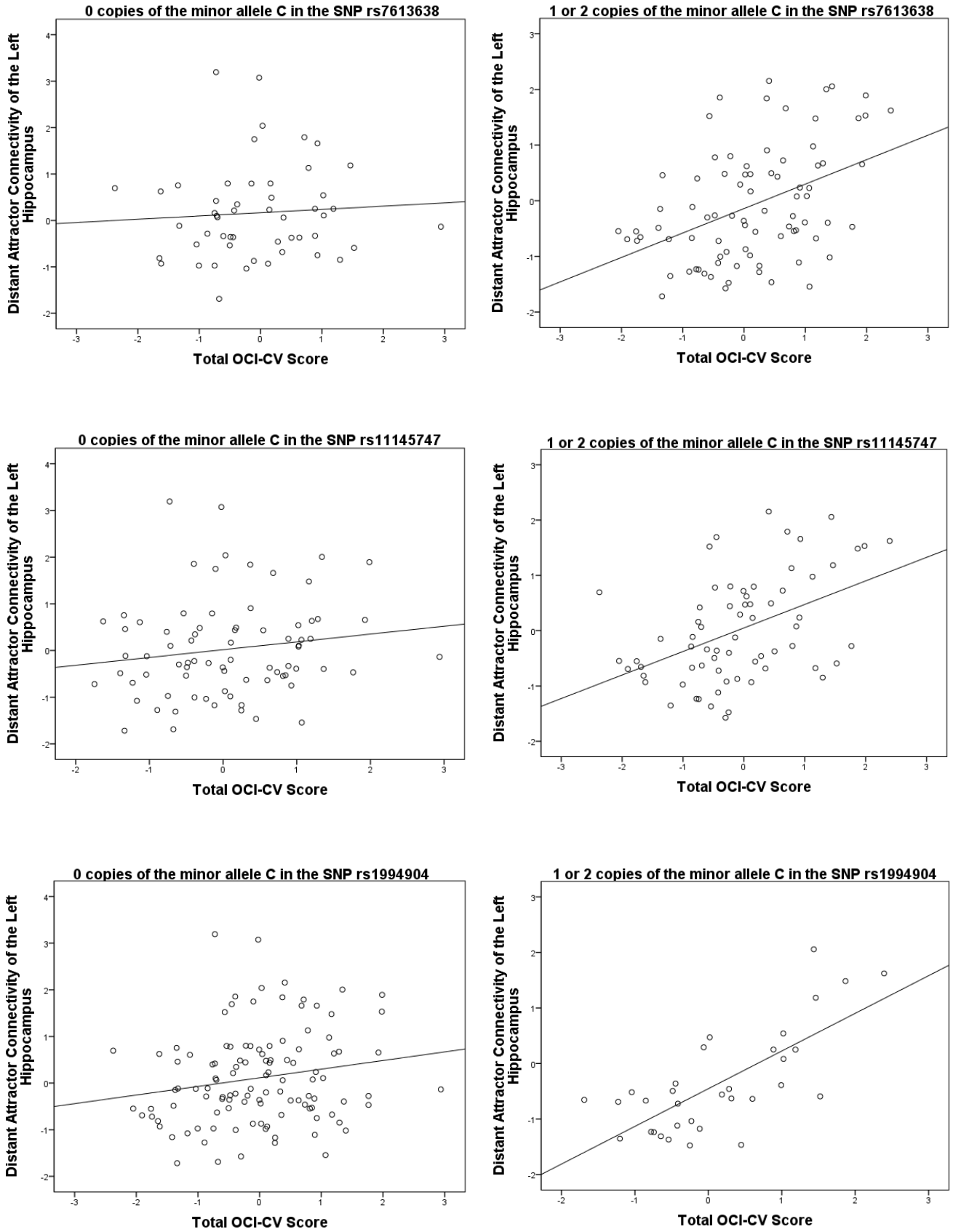


**Figure S1**: Scatter plots of the moderator effect of the SNPs rs7613638, rs11145747 and rs1994904 on the correlation between total OCI-CV score and distant attractor connectivity of the left hippocampus. OCI-CV: Obsessive Compulsive Inventory-Child Version; SNP: Single-Nucleotide Polymorphism.

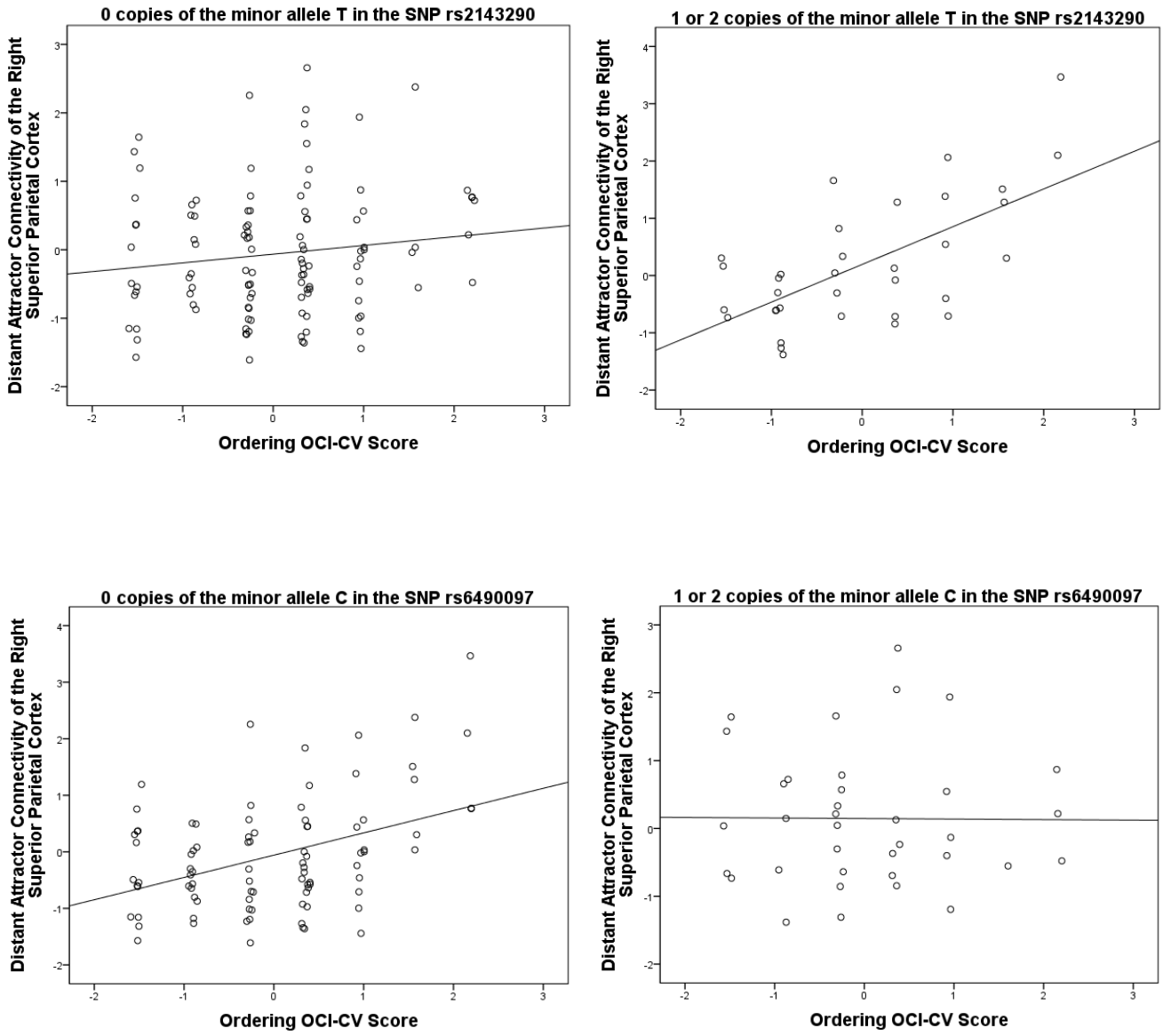


**Figure S2**: Scatter plots of the moderator effect of the SNPs rs2143290 and rs6490097 on the correlation between ordering OCI-CV score and distant attractor connectivity of the right superior parietal cortex. OCI-CV: Obsessive Compulsive Inventory-Child Version; SNP: Single-Nucleotide Polymorphism.

**Table S1**: Comparison of OCI-CV scores between the study sample and the Spanish validation sample (Rosa-Alcázar et al., 2014)

| **Obsessive Compulsive Inventory-Child Version (OCI-CV)** | | |
|---|---|---|
| **Our project** | **N=227 (8-12y)** | |
| | *Mean (SD)* | *Median* |
| Doubting-Checking | 2.25 (1.52) | 2 |
| Hoarding | 2.04 (1.26) | 2 |
| Neutralizing | 0.38 (0.72) | 0 |
| Obsessing | 1.59 (1.40) * | 1 |
| Ordering | 2.23 (1.60) | 2 |
| Washing | 0.59 (0.82) * | 0 |
| Total | 9.08 (4.08) * | 9 |
| **Spanish Validation Sample** | **N=914 (8-18y)** | |
| | *Mean (SD)* | *Median* |
| Doubting-Checking | 2.13 (1.64) | - |
| Hoarding | 1.90 (1.35) | - |
| Neutralizing | 0.32 (0.72) | - |
| Obsessing | 2.39 (2.01) | - |
| Ordering | 2.20 (1.68) | - |
| Washing | 3.10 (2.16) | - |
| Total | 11.34 (6.09) | - |

*Denotes a score significantly different from the score of the Spanish validation sample ($p<0.05$, one-sample t-test).

**Table S2**: Comparison of SDQ scores between the study sample and the Spanish validation sample (Barriuso-Lapresa et al., 2014)

| **Strengths and Difficulties Questionnaire (SDQ)** | | |
|---|---|---|
| **Our project** | **N=222 (8-12y)** | |
| | *Mean (SD)* | *Median* |
| Emotional Symptoms (0-10) | 1.88 (1.78) | 1 |
| Conduct Problems (0-10) | 1.58 (1.54) * | 1 |
| Hyperactivity/Inattention (0-10) | 3.72 (2.39) * | 4 |
| Peer Relationship Problems (0-10) | 1.09 (1.38) * | 1 |
| Total Difficulties Score (0-40) | 8.27 (4.90) * | 8 |
| Prosocial Behavior (0-10) | 8.69 (1.57) | 9 |
| **Spanish Validation Sample** | **N=6266 (34.10% aged 8-11y)** | |
| | *Mean (SD)* | *Median* |
| Emotional Symptoms (0-10) | 2.10 (0.06) | 2 |
| Conduct Problems (0-10) | 1.87 (0.05) | 1 |
| Hyperactivity/Inattention (0-10) | 4.19 (0.08) | 4 |
| Peer Relationship Problems (0-10) | 1.32 (0.05) | 1 |
| Total Difficulties Score (0-40) | 9.49 (0.18) | 9 |
| Prosocial Behavior (0-10) | 8.81 (0.04) | 10 |

*Denotes a score significantly different from the score of the Spanish validation sample ($p<0.05$, one-sample t-test).

**Table S3**: Genes significantly associated with distinct attractor connectivity maps

| Total symptom-related subcortical distant attractor map **(hippocampus L)** | | | | Total symptom-related cortical distant attractor map **(SMA R/L)** | | Total symptom-related subcortical local attractor map **(vent Putamen L)** | Ordering symptom-related cortical distant attractor map **(Sup Parietal R)** | | |
|---|---|---|---|---|---|---|---|---|---|
| ACCN1 | YWHAB | FGFR1OP2 | C7orf70 | ALX3 | PECR | SLC25A6 | ACVRL1 | DGKI | ZKSCAN3 |
| ACVR1 | YWHAG | AK5 | AGBL4 | AMD1 | ANKH | CHD1 | ADARB1 | DCLK1 | PRR3 |
| ACVR2A | YWHAH | NBEA | LINGO1 | ATP1B1 | SERTAD4 | ERCC5 | ACAN | XPR1 | SETD7 |
| ADCY2 | YWHAZ | RNF11 | LRP11 | CALM2 | RP5-1022P6.1 | GLUL | ALDH1B1 | ZFYVE9 | KCNH6 |
| AP2A2 | ZNF711 | KCNV1 | ZNRF1 | CDS1 | EIF5A2 | PPP1R12B | ANK1 | MED21 | OR51E2 |
| ANK2 | ZNF184 | FILIP1 | C3orf34 | CEACAM7 | THAP10 | PTPRB | ANXA13 | EEF1E1 | FCRL5 |
| APC | ZNF215 | GPR77 | ZNF518B | EGR1 | PMEPA1 | RBL2 | STS | MRPL33 | RASSF5 |
| ARF1 | SLC30A3 | BHLHE22 | NAV3 | ESRRA | CEACAM19 | ZFY | ATP1B1 | CLOCK | CD99L2 |
| ASTN1 | CUL5 | PCLO | C16orf45 | ESRRG | HYMAI | ALMS1 | ATP2A2 | ENTPD4 | ROPN1L |
| ATP6V1C1 | CDC7 | NKIRAS1 | UNC5A | F7 | AS3MT | BHLHE40 | ATP2B1 | SRGAP3 | TTC29 |
| ATP6V1E1 | PIP5K1A | TMOD2 | CCDC109A | FGF9 | NDRG3 | PER3 | ATP2B2 | HS3ST1 | EMILIN2 |
| BDNF | PIP5K2B | C2orf27A | LONRF1 | FLT3 | HEG1 | CASP8AP2 | ATP2B3 | RWDD2B | LRRC8C |
| BMPR2 | DOC2A | GRHL1 | ZNF804A | GABRA1 | AADACL1 | RBM14 | BCAT1 | CACNG2 | C14orf142 |
| ERC2-IT1 | RAE1 | UBQLN2 | LMBRD2 | GABRG2 | STIM2 | CNOT1 | BICD1 | CITED2 | KIAA1841 |
| MPPED1 | MAP4K3 | TAS2R9 | MOBKL1A | GAPDH | WDR19 | RRP1B | BID | SLC19A2 | NT5C1A |
| CACNA2D1 | PPFIA2 | GLOD4 | HNRPLL | GAS6 | CCNB1IP1 | SMC5 | CACNB4 | SMC2 | GLIS2 |
| CALM3 | ENC1 | PI15 | CADPS2 | GNB3 | SLC22A23 | RP1-21O18.1 | CALML3 | POSTN | AGPAT9 |
| CAMK2A | LMO4 | ATL1 | TP53RK | GPLD1 | UBE2O | SIRT1 | CDH6 | SPHAR | ATAD1 |
| CCK | CHRD | POLR1D | LACTB | NR3C1 | DUS1L | NIPBL | CDS1 | CUGBP2 | MASTL |
| CCKBR | B3GALT1 | ADIPOR1 | XKR4 | GTF2A1 | LMBR1 | DSE | CEBPE | GRAP | SERAC1 |
| CCNC | NOL4 | TRNT1 | GPRIN1 | GYS1 | CERK | TAOK3 | CHRM2 | PDE10A | C3orf34 |
| CD36 | ADAM20 | CCDC41 | CSMD3 | IFI27 | GNPNAT1 | MBTD1 | COL19A1 | DBF4 | USP45 |
| CDH2 | MTMR1 | HN1 | NUS1 | IMPA1 | RANBP17 | NOL8 | CPN2 | TBC1D8 | FHDC1 |
| CDH10 | TRIM24 | HSPA14 | DACH2 | INPP5A | FASTKD1 | GPATCH1 | CS | KLF12 | ARHGAP11B |
| CHD3 | CDKL1 | RAPGEFL1 | TRAPPC6B | IPW | ASB13 | PRPF38B | DLSTP | POU6F2 | CCDC126 |
| CHGB | LIN7A | CXorf26 | NAA30 | IRS1 | TTC21B | ZCCHC8 | EDNRA | CBX3 | ZNF697 |
| CHN1 | TSC22D1 | MRPS17 | TTC8 | JARID2 | MAP9 | UBAP2 | EGR1 | HSPA4L | PLXNA4 |
| AP3S1 | CDK5R1 | ATP6V1D | HIGD2B | KCNA1 | L2HGDH | MLL5 | ELAVL2 | SLITRK3 | SYT12 |
| CCR6 | CCNA1 | PPME1 | CDYL2 | MCF2 | EDEM3 | COG6 | ELAVL4 | INPP5F | RP11-82I10.1 |
| CNTN1 | BSN | HPCAL4 | TTC39C | MGST2 | SETD7 | KDM2B | EPHA1 | PLA2R1 | SCGB3A1 |
| ATF2 | WASL | NT5DC3 | ZNF441 | NR3C2 | CDADC1 | CALML4 | ERG | SV2C | TIFA |
| CRMP1 | USP14 | MED15 | TNFAIP8L1 | MPP1 | C18orf21 | C1QTNF3 | ERN1 | TTC39A | TMEM169 |
| CSNK1G3 | MTMR6 | ATP6V1H | FAM148C | NARS | CD99L2 | AGAP1 | ESRRG | TRIM35 | HELB |
| DMD | DCLK1 | TMEM66 | C1orf216 | PAK2 | SLC4A9 | XRRA1 | EXTL2 | CLASP2 | RG9MTD2 |
| DNM1 | LRRFIP1 | SPA17 | VSTM2L | PCP4 | FAM57B | SLC2A12 | F7 | PASK | CADPS2 |

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|---|---|---|---|
| DYRK1A | DLGAP1 | LINC00158 | LYZL4 |
| TOR1A | NUMBL | GNG2 | TMEM155 |
| EFNB1 | TCEAL1 | GDAP1 | SPATA18 |
| CELSR3 | CPNE6 | TAF7L | STXBP5 |
| ENSA | NRXN3 | TOLLIP | C6orf57 |
| ETS2 | PLAA | TMX3 | RP11-63L7.1 |
| EXTL1 | NRXN1 | EXOC6 | ZNF786 |
| FABP7 | COG1 | ARL15 | C7orf57 |
| ACSL4 | IL27RA | PALMD | UBXN2B |
| FLOT2 | CABP1 | TMEM160 | SLITRK4 |
| GABRB3 | SLC4A7 | FEZF2 | MUM1L1 |
| B4GALNT1 | PAGE4 | GPATCH2 | ZFP28 |
| GDI1 | BAG4 | AKIRIN2 | SIRPA |
| GLRB | BRE | ARL8B | LRFN5 |
| GLS | PREPL | TMEM57 | FAM81A |
| GNAQ | RAB36 | PNMAL1 | IL34 |
| GNG10 | GDA | PSPC1 | TTC9B |
| GPR22 | MICAL2 | MCOLN3 | C1orf52 |
| GRIA1 | PHF14 | UBE2W | MFSD4 |
| GRIA2 | RAPGEF2 | FBXW7 | FAM84A |
| GRIK2 | PUM1 | RFK | UPP2 |
| GRM7 | KIAA0513 | LIN7C | C4orf45 |
| GYPB | MATR3 | SVOP | PPP1R2P3 |
| GYPE | IP6K1 | UBE2Q1 | C6orf165 |
| HIST1H1A | ARMCX2 | CCDC132 | C7orf60 |
| HMGCR | DNAJC6 | TASP1 | PRSS2 |
| HPRT1 | PJA2 | LRRC40 | TMEM74 |
| HRH1 | RP4-788L13.1 | AC079061.1 | ZNF483 |
| HTR1A | SNAP91 | PARVA | TMTC3 |
| IDS | SV2B | SCN3B | ZFP1 |
| KCND3 | G3BP2 | ZC3H15 | ZNF433 |
| KCNG1 | FAM20B | BEX1 | PPAPR5 |
| KPNA3 | MAMLD1 | MESP1 | FLJ39061 |
| LGALS8 | ATP6AP2 | CMAS | C1QL2 |
| FADS3 | RRAGB | LANCL2 | FAM151B |
| LY6H | MYCNOS | SEPT3 | TXLNB |
| M6PR | OLFM1 | PCDHB11 | ZCCHC12 |
| MAP2 | FAM3C | PCDHAC2 | C12orf28 |
| MIPEP | ZNF238 | BEX4 | CREG2 |
| MLLT3 | SLC9A6 | NPDC1 | C17orf108 |
| PPP1R12A | CAP2 | BRUNOL4 | RAB12 |
| NAP1L2 | SEMA4F | MEIS3P2 | USP12 |
| NCAM2 | DEAF1 | ARNTL2 | MED19 |
| NDUFA5 | CHERP | CDC42SE2 | DOK6 |
| NEK2 | SSSCA1 | SLC17A7 | ATOH7 |

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|---|---|
| PDC | CTTNBP2 |
| SERPINB9 | ARID5B |
| PLAGL1 | TRAF7 |
| UBL3 | KIAA1841 |
| PRKACB | C1orf97 |
| RBMS1 | ZNF496 |
| RFX5 | SLC35B4 |
| RORA | PPP1R15B |
| SCN1A | CORO6 |
| SCN4B | FHDC1 |
| SGCG | C1orf201 |
| SIAH2 | LOC90834 |
| SLC8A1 | ESAM |
| ELOVL4 | TMEM88 |
| VAMP1 | TIFA |
| TPP2 | OSBPL6 |
| UBE2D1 | FLYWCH2 |
| UPP1 | MOGAT1 |
| ZNF133 | JDP2 |
| SEMA7A | WDR63 |
| SLC25A12 | SYT2 |
| SUCLA2 | CMPK2 |
| DCLK1 | CNTNAP5 |
| ITM2A | C4orf33 |
| ROCK2 | PIP5KL1 |
| KIF23 | ANKRD29 |
| SLC4A8 | ZNF362 |
| STXBP5L | LOC149837 |
| MRPL33 | RP11-403C10.2 |
| PREPL | LOC158696 |
| SPOCK2 | STRC |
| TBC1D4 | C19orf46 |
| SV2A | GBP6 |
| ABCA10 | FLJ23867 |
| SLC19A2 | SH3D20 |
| SMC2 | CMYA5 |
| MASP2 | BEND6 |
| TBC1D8 | RICTOR |
| HSPA4L | TMEM86B |
| SLITRK3 | C4orf22 |
| PLEKHA6 | PTF1A |
| TTC39A | LOC256374 |
| USP33 | FAM133B |
| SMG1 | TAS2R45 |
| LRCH1 | OR2L13 |

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|---|
| SYCP2L |
| GK5 |
| WHAMML1 |
| C6orf26 |
| ZNF506 |
| LOC730234 |
| LOC731275 |
| DCAF13P3 |
| AC133485.2 |

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|---|---|---|
| FGF5 | SIK2 | RP1L1 |
| FGF9 | PLCB1 | FOXQ1 |
| FGF10 | ACSL6 | RNF157 |
| FLT3 | TBC1D30 | OSBPL6 |
| GABRA1 | SULT4A1 | TM4SF18 |
| GABRG2 | BRP44 | FAT3 |
| GABRG3 | C2CD3 | TC2N |
| GAS2 | OSBPL3 | MTFMT |
| GK | CCDC69 | NIPA1 |
| GNGT2 | SACS | ZNF491 |
| GSTA1 | CHORDC1 | SYT2 |
| HAS3 | NPTN | AP1S3 |
| NRG1 | TSPAN13 | CPNE4 |
| HLF | NOB1 | STARD4 |
| HOXA7 | RP5-1068E13.3 | OR13C8 |
| HTR1B | CD274 | DYNLL2 |
| IDH3A | C2orf27A | C20orf173 |
| IFIT1 | ST8SIA5 | PRICKLE1 |
| IL7R | NME7 | CCDC64B |
| IL13 | TRHDE | TRIM16L |
| INHBA | HUNK | C18orf25 |
| INPP5A | ZNF295 | ANKRD29 |
| INSL3 | SOST | PDIK1L |
| IRS1 | MED31 | LOC149837 |
| ISLR | GLRX2 | SMYD1 |
| KCNA1 | VPS36 | CNTN4 |
| KCNA2 | ST8SIA3 | SHISA3 |
| KCNA3 | TUBE1 | SLC36A2 |
| KCNC1 | GP6 | ALDH1L2 |
| KCNS3 | TBC1D7 | C15orf26 |
| LAMA5 | IER5 | PGBD4 |
| LGALS8 | GCNT4 | GBP6 |
| LMNB1 | UCHL5 | KANK4 |
| MCF2 | FAM49B | SYNPO2 |
| MGAT5 | CAB39 | SCAMP5 |
| MGST2 | PANK1 | FLJ23867 |
| TRIM37 | GPR84 | ANO5 |
| MYC | GPR85 | C7orf62 |
| MYL4 | MAGEL2 | ZNF804B |
| NAP1L2 | AC131238.1 | GJD4 |
| NEFH | MANSC1 | SPATA19 |
| NSF | A2BP1 | RNF152 |
| AP000843.1 | LRRC49 | VSTM2A |
| P2RX3 | WDR55 | NKAPL |
| PCP4 | KIAA1797 | C4orf22 |

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|---|---|---|---|
| NELL2 | RPP30 | MAN1C1 | RSPH9 |
| NEUROD1 | SCGN | TRIM54 | AOF1 |
| NEUROD2 | CDC42EP3 | SLC39A10 | JAZF1 |
| NOV | AC011479.2 | SLC4A10 | TMEM130 |
| NPPA | PNMA2 | RHBDD2 | RNASEH1 |
| NPTX1 | CCT8 | PLEKHG5 | LASS6 |
| NPTX2 | TBR1 | ODZ2 | YTHDF3 |
| NPY2R | CHL1 | ODF2L | NEGR1 |
| NSF | PGRMC1 | PDCD6 | TIPRL |
| OCM2 | TCERG1 | KIAA1239 | PGM2L1 |
| CNTN3 | SUB1 | MTA3 | GAS2L3 |
| PCDH7 | SLC27A2 | TMEM181 | MIR7-3HG |
| SLC26A4 | PIM2 | ZBTB2 | DNAJC5G |
| PFN2 | RAPGEF4 | BEND3 | FAM126B |
| PKP2 | WDR5 | MAGEE1 | EPHA6 |
| PLXNA1 | KIF3A | DENND1A | C3orf33 |
| PPARG | CORO1A | PPP4R4 | AC021850.1 |
| PPAT | DDX20 | WDR19 | ARL10 |
| PPL | RNF24 | C1orf114 | AC091878.1 |
| PPP2CA | NXPH2 | C6orf115 | RGMB |
| PPP2R5E | RRAS2 | PKNOX2 | LY86-AS1 |
| PPP3CB | NLGN4Y | NAPB | AC078937.4 |
| PRKAR1B | ZNF510 | ZNF667 | EFHA2 |
| PRKCE | NLGN1 | CLSPN | FAM19A2 |
| PRKCI | ANKRD6 | NEUROD6 | MYLK4 |
| MAPK9 | WDR47 | CLSTN2 | KIAA2022 |
| PRSS1 | MAPRE3 | AC093283.3 | BEX5 |
| PRSS2 | SEPHS2 | WBSCR17 | KCNT2 |
| PRSS3 | SHANK2 | CSMD1 | HCN1 |
| KLK7 | RIMS1 | AC024575.2 | SLCO4C1 |
| PTH2R | FBXO21 | USP46 | ZNF699 |
| PTPRG | DOPEY1 | OBFC2A | LOC375196 |
| PTPRO | PHLPP2 | PAPOLG | C6orf173 |
| PTPRR | KIF21B | CASD1 | RP11-45B20.3 |
| RAB2A | PDS5B | MRPL1 | TNFAIP8L3 |
| RAB27B | MYCBP2 | RAPH1 | C16orf87 |
| RAP2B | AVL9 | SPATS2 | C2orf80 |
| RARRES1 | ARHGAP26 | GDAP1L1 | C1QL1 |
| RASA1 | MRPS27 | C1orf135 | C13orf36 |
| RASA2 | GARNL4 | PRRG3 | FAM174B |
| ARID4A | RTF1 | C9orf16 | MIR2682 |
| RFC3 | CEP68 | LRRC2 | AC112641.2 |
| RNF2 | RFTN1 | PGBD5 | LOC401533 |
| SCN2A | ARL6IP1 | NIPAL2 | C16orf74 |
| MAP2K4 | VPS13A | ARHGAP28 | FAM19A1 |

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|---|---|
| EFR3A | INTS4L1 |
| KIAA0182 | SPDYE1 |
| MCF2L | XKR6 |
| MTUS2 | EFHA2 |
| TBC1D30 | RAB37 |
| ZFPM2 | C3orf35 |
| CAPN7 | AGBL3 |
| RAB3GAP2 | STAC2 |
| AC110760.2 | SLC6A10P |
| KLHL3 | ANKRD34C |
| CHORDC1 | RP4-695O20__B.1 |
| PKD2L2 | IQSEC3 |
| C12orf24 | CKMT1A |
| TAS2R9 | TMPPE |
| ZDHHC2 | LOC645722 |
| GPR89B | LOC646278 |
| GPR85 | AC135983.8 |
| MAGEL2 | FAM155A |
| LRRC49 | A26C1B |
| CCDC40 | RP11-422J8.1 |
| DCUN1D2 | GOLGA8C |
| BBS7 | AC011511.1 |
| RNLS | LOC100131471 |
| STRBP | LOC100286909 |
| BCAS4 | LOC100288939 |

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|---|---|---|
| PENK | TESC | PTF1A |
| PFTK1 | EPN3 | MAP3K7IP3 |
| PIK3CB | KLHL11 | FLJ33996 |
| PIM1 | LGI2 | CDRT4 |
| PLA2G4A | C7orf43 | PIGW |
| PLCB4 | UBE2W | KCTD1 |
| POU3F3 | TBC1D19 | ZNF547 |
| POU3F4 | RNLS | OR2L13 |
| PPP3CA | STRBP | CYB561D1 |
| PRKAR2A | ABHD10 | RIMKLA |
| PRKCB | YOD1 | FAM126B |
| PTGS2 | C2orf29 | SPDYE1 |
| PVALB | MREG | EFHA2 |
| PEX5 | FAR2 | FAM78A |
| RASGRF1 | C17orf63 | RAB37 |
| RET | HHAT | RNASE10 |
| RNASEL | PECR | CCDC39 |
| RNF6 | SLC25A40 | C12orf70 |
| RPS6KA3 | PCDHGA1 | FMN1 |
| CLEC11A | PCDHB15 | STAC2 |
| SCN1A | PCDHA3 | PAQR9 |
| SCN1B | ANKH | HCN1 |
| SCN4B | LMOD3 | FKBP9L |
| SRL | SERTAD4 | TPTE2P6 |
| SDC1 | ASAH2 | VWC2 |
| SETMAR | EIF5A2 | RNF148 |
| SCG5 | PMEPA1 | RASL11A |
| SH3GL2 | PRDM8 | VSTM5 |
| SLC5A4 | SNX14 | SRP14P1 |
| SLC6A6 | NDRG3 | ANKRD34C |
| SLC20A1 | KCTD16 | OR2AK2 |
| SNAP25 | AADACL1 | MSLNL |
| SOX15 | KCNT1 | VWC2L |
| ELOVL4 | PCDHB16 | AC090360.1 |
| VAMP1 | DMRT3 | CISD2 |
| TEAD4 | ENPP5 | MIR31HG |
| TGM1 | CACNG8 | INTS4L2 |
| TCHH | TMEM35 | LOC646548 |
| TRAF5 | FASTKD5 | LOC646976 |
| TRPC5 | LMBR1 | ZNF732 |
| AC087742.2 | ARHGAP9 | FAM155A |
| ZNF41 | IL25 | RP11-422J8.1 |
| ZNF133 | GNPNAT1 | ZBTB8OSP1 |
| SLC25A16 | RFX7 | RP1-152L7.5 |
| MADCAM1 | STAG3L4 | LOC730167 |

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|---|---|---|---|
| SH3GL2 | RPRD2 | NRSN2 | MRC1L1 |
| ST3GAL1 | TSPYL5 | PANK2 | FAM116B |
| SLA | CAMSAP1L1 | CSRNP3 | CXADRP3 |
| SLC3A1 | DMXL2 | PGAP1 | UNC13C |
| SLCO2A1 | USP22 | BAIAP2L2 | NUDT4P1 |
| SLIT1 | WDR7 | TM2D3 | TMEM150C |
| SLIT3 | KIAA1045 | WDR82 | IGFL4 |
| SMARCA2 | KIAA0895 | NDFIP1 | AC068491.1 |
| SNAP25 | PPP1R13B | CYB5B | FAM110C |
| SSTR1 | KIAA0947 | CSRNP2 | AC136443.1 |
| STX1A | ZFPM2 | ACSBG2 | FAM25A |
| STXBP1 | NPTXR | PPP1R14C | LOC644189 |
| SYN1 | RIMBP2 | ST6GALNAC5 | RLIMP3 |
| SYN2 | NNT | ARPC5L | AC016683.6 |
| SYT1 | CCRK | SEH1L | KRTAP4-8 |
| TG | LEMD3 | DNAL1 | LOC728392 |
| KLF10 | LDOC1 | C19orf12 | LOC728673 |
| TRPC1 | SSBP3 | ARL6 | C12orf71 |
| TRPC4 | GSPT2 | AC113191.1 | LOC100127925 |
| TSPYL1 | ATXN10 | ZNF541 | RP11-244F12.3 |
| TTC3 | CCRN4L | CHCHD6 | LOC100129291 |
| UBE2B | AC109486.1 | HOPX | LOC100133211 |
| UBE3A | CCDC28A | C21orf67 | AP000880.1 |
| SUMO1 | MYRIP | CCDC62 | ERLEC1P1 |
| VIP | CNRIP1 | BEX2 | LOC100288929 |
| XK | ERC2 | BAT2L | LOC100289671 |
| XRCC5 | | | |

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|---|---|---|
| UTF1 | RTN4R | LOC731602 |
| SEMA7A | ELOVL6 | HAR1A |
| BLZF1 | C6orf211 | C20orf78 |
| SLC25A12 | C16orf57 | RP5-1027G4.3 |
| CADPS | ZNF385D | AC009139.1 |
| ABCC3 | ZFAND1 | AC002351.1 |
| NAPA | NIPAL2 | LOC100133686 |
| SUCLA2 | AP000926.2 | LOC100287813 |
| LIN7A | MAP9 | LOC100289350 |
| TMEM11 | WDR76 | MCF2L-AS1 |
| CDKL2 | VCPIP1 | LOC100290397 |
| MTMR4 | C14orf45 | C21orf37 |
| INA | MOGAT2 | |

**Table S4**: Gene Ontology over-representation analysis of genes associated with the total symptom-related subcortical distant attractor connectivity map

| GO biological process | Fold Enrichment | Raw P-value | FDR |
|---|---|---|---|
| glutamine catabolic process | 35.22 | 3.93E-04 | 3.88E-02 |
| synaptic vesicle clustering | 19.57 | 2.57E-05 | 4.30E-03 |
| neuron cell-cell adhesion | 14.50 | 2.71E-06 | 6.25E-04 |
| positive regulation of synaptic vesicle recycling | 14.09 | 4.62E-04 | 4.32E-02 |
| regulation of synaptic vesicle endocytosis | 11.74 | 3.80E-05 | 5.92E-03 |
| glutamate secretion | 11.36 | 1.24E-07 | 4.18E-05 |
| synaptic vesicle priming | 11.01 | 2.22E-04 | 2.47E-02 |
| regulation of clathrin-dependent endocytosis | 9.78 | 3.51E-04 | 3.56E-02 |
| regulation of synaptic vesicle recycling | 9.48 | 2.70E-05 | 4.47E-03 |
| maintenance of synapse structure | 9.27 | 4.34E-04 | 4.13E-02 |
| postsynapse assembly | 9.27 | 4.34E-04 | 4.10E-02 |
| synaptic vesicle localization | 8.81 | 2.57E-07 | 8.02E-05 |
| presynapse organization | 8.13 | 2.12E-04 | 2.44E-02 |
| ionotropic glutamate receptor signaling pathway | 8.13 | 2.12E-04 | 2.43E-02 |
| behavioral fear response | 7.95 | 7.07E-05 | 1.01E-02 |
| behavioral defense response | 7.70 | 8.42E-05 | 1.15E-02 |
| calcium-ion regulated exocytosis | 7.55 | 9.48E-06 | 1.84E-03 |
| fear response | 7.47 | 9.96E-05 | 1.34E-02 |
| regulation of synaptic vesicle cycle | 7.16 | 1.07E-12 | 2.44E-09 |
| postsynaptic membrane organization | 7.04 | 4.15E-04 | 4.02E-02 |
| synaptic transmission, glutamatergic | 7.04 | 4.15E-04 | 4.00E-02 |
| presynaptic endocytosis | 6.77 | 7.04E-06 | 1.49E-03 |
| synaptic vesicle endocytosis | 6.77 | 7.04E-06 | 1.47E-03 |
| acidic amino acid transport | 6.77 | 7.04E-06 | 1.45E-03 |
| axon extension | 6.66 | 1.86E-04 | 2.29E-02 |
| glutamate receptor signaling pathway | 6.60 | 2.42E-05 | 4.09E-03 |
| synaptic vesicle transport | 6.49 | 2.15E-04 | 2.44E-02 |
| synaptic vesicle cycle | 6.43 | 2.21E-11 | 2.92E-08 |
| positive regulation of amine transport | 6.32 | 2.48E-04 | 2.68E-02 |
| synaptic vesicle exocytosis | 6.05 | 6.40E-06 | 1.39E-03 |
| neurotransmitter secretion | 6.04 | 7.99E-09 | 3.85E-06 |
| signal release from synapse | 6.04 | 7.99E-09 | 3.73E-06 |
| vesicle-mediated transport in synapse | 5.96 | 8.55E-11 | 9.71E-08 |
| receptor clustering | 5.87 | 1.42E-04 | 1.84E-02 |
| synaptic vesicle recycling | 5.87 | 2.13E-05 | 3.68E-03 |
| neuron recognition | 5.64 | 1.83E-04 | 2.27E-02 |
| regulation of synaptic vesicle exocytosis | 5.52 | 2.35E-06 | 5.58E-04 |
| modulation of excitatory postsynaptic potential | 5.48 | 5.37E-04 | 4.96E-02 |
| vesicle docking | 4.95 | 1.77E-04 | 2.23E-02 |
| dicarboxylic acid transport | 4.90 | 3.74E-05 | 5.89E-03 |

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|---|---|---|---|
| neuron projection extension | 4.80 | 2.18E-04 | 2.45E-02 |
| neurotransmitter transport | 4.73 | 4.82E-08 | 1.87E-05 |
| regulation of neurotransmitter receptor activity | 4.56 | 6.83E-05 | 9.87E-03 |
| regulation of neurotransmitter secretion | 4.48 | 8.58E-06 | 1.68E-03 |
| exocytic process | 4.46 | 3.56E-04 | 3.58E-02 |
| regulation of synaptic transmission, glutamatergic | 4.40 | 3.90E-04 | 3.88E-02 |
| regulation of neurotransmitter transport | 4.23 | 7.87E-06 | 1.56E-03 |
| developmental cell growth | 4.21 | 1.30E-04 | 1.69E-02 |
| cell growth | 4.12 | 1.54E-04 | 1.98E-02 |
| regulation of pH | 4.10 | 8.14E-05 | 1.13E-02 |
| regulation of intracellular pH | 4.05 | 3.48E-04 | 3.56E-02 |
| localization within membrane | 3.96 | 4.10E-04 | 4.00E-02 |
| signal release | 3.80 | 3.43E-07 | 1.05E-04 |
| regulation of neurotransmitter levels | 3.78 | 3.19E-08 | 1.30E-05 |
| synapse assembly | 3.76 | 3.19E-04 | 3.29E-02 |
| regulation of exocytosis | 3.72 | 1.41E-07 | 4.68E-05 |
| negative regulation of dephosphorylation | 3.68 | 2.11E-04 | 2.46E-02 |
| regulation of synaptic plasticity | 3.65 | 2.05E-06 | 5.01E-04 |
| chemical synaptic transmission | 3.63 | 3.27E-12 | 5.77E-09 |
| anterograde trans-synaptic signaling | 3.63 | 3.27E-12 | 5.19E-09 |
| modulation of chemical synaptic transmission | 3.60 | 4.16E-13 | 1.65E-09 |
| regulation of trans-synaptic signaling | 3.59 | 4.47E-13 | 1.42E-09 |
| negative regulation of phosphatase activity | 3.59 | 4.62E-04 | 4.30E-02 |
| regulation of regulated secretory pathway | 3.59 | 1.46E-05 | 2.56E-03 |
| synaptic signaling | 3.56 | 5.93E-13 | 1.57E-09 |
| trans-synaptic signaling | 3.55 | 3.52E-12 | 5.08E-09 |
| vesicle localization | 3.52 | 1.93E-06 | 4.79E-04 |
| negative regulation of cell projection organization | 3.46 | 1.27E-05 | 2.29E-03 |
| negative regulation of neuron projection development | 3.45 | 6.83E-05 | 9.96E-03 |
| amino acid transport | 3.44 | 2.11E-04 | 2.45E-02 |
| regulation of circadian rhythm | 3.44 | 3.71E-04 | 3.71E-02 |
| learning | 3.31 | 1.78E-04 | 2.23E-02 |
| regulation of nervous system process | 3.29 | 1.90E-04 | 2.32E-02 |
| axonogenesis | 3.23 | 3.51E-09 | 2.15E-06 |
| axon development | 3.21 | 9.46E-10 | 7.91E-07 |
| axon guidance | 3.14 | 1.42E-06 | 3.70E-04 |
| neuron projection guidance | 3.13 | 1.51E-06 | 3.86E-04 |
| cell morphogenesis involved in neuron differentiation | 3.08 | 1.68E-09 | 1.27E-06 |
| learning or memory | 3.05 | 6.08E-06 | 1.34E-03 |
| establishment of vesicle localization | 2.99 | 1.18E-04 | 1.56E-02 |
| regulation of phosphatase activity | 2.97 | 3.17E-04 | 3.32E-02 |
| regulation of axonogenesis | 2.94 | 2.28E-04 | 2.52E-02 |
| neuron projection morphogenesis | 2.92 | 2.80E-09 | 1.86E-06 |
| regulation of signaling receptor activity | 2.90 | 3.95E-04 | 3.88E-02 |

| | | | |
|---|---|---|---|
| plasma membrane bounded cell projection morphogenesis | 2.90 | 3.48E-09 | 2.21E-06 |
| regulation of vesicle-mediated transport | 2.89 | 6.54E-10 | 5.78E-07 |
| cell projection morphogenesis | 2.88 | 4.30E-09 | 2.44E-06 |
| regulation of endocytosis | 2.86 | 1.92E-04 | 2.33E-02 |
| cognition | 2.85 | 7.14E-06 | 1.45E-03 |
| cell part morphogenesis | 2.83 | 4.64E-09 | 2.54E-06 |
| locomotory behavior | 2.82 | 3.49E-04 | 3.55E-02 |
| regulation of dephosphorylation | 2.81 | 2.37E-04 | 2.59E-02 |
| negative regulation of neuron differentiation | 2.81 | 1.61E-04 | 2.04E-02 |
| protein localization to cell periphery | 2.78 | 1.21E-04 | 1.58E-02 |
| synapse organization | 2.76 | 2.70E-05 | 4.42E-03 |
| regulation of neuron projection development | 2.70 | 4.95E-08 | 1.87E-05 |
| neuron development | 2.70 | 2.64E-12 | 5.24E-09 |
| neuron projection development | 2.68 | 4.14E-10 | 4.11E-07 |
| cell morphogenesis involved in differentiation | 2.67 | 2.24E-08 | 9.63E-06 |
| regulation of synapse structure or activity | 2.64 | 3.18E-04 | 3.31E-02 |
| regulation of cell projection organization | 2.54 | 5.15E-09 | 2.64E-06 |
| cellular component morphogenesis | 2.54 | 4.54E-08 | 1.81E-05 |
| regulation of plasma membrane bounded cell projection organization | 2.52 | 8.91E-09 | 4.05E-06 |
| cell morphogenesis | 2.52 | 4.75E-09 | 2.51E-06 |
| regulation of membrane potential | 2.48 | 9.92E-06 | 1.81E-03 |
| behavior | 2.46 | 3.63E-07 | 1.09E-04 |
| neuron differentiation | 2.38 | 6.05E-11 | 7.39E-08 |
| regulation of neuron differentiation | 2.34 | 3.93E-07 | 1.16E-04 |
| organelle localization | 2.27 | 6.52E-06 | 1.40E-03 |
| generation of neurons | 2.24 | 9.79E-14 | 7.78E-10 |
| regulation of cell morphogenesis | 2.22 | 7.88E-05 | 1.12E-02 |
| cell junction organization | 2.21 | 8.07E-05 | 1.12E-02 |
| protein localization to membrane | 2.19 | 6.35E-05 | 9.35E-03 |
| neurogenesis | 2.19 | 1.48E-13 | 7.83E-10 |
| chemotaxis | 2.18 | 4.87E-05 | 7.52E-03 |
| taxis | 2.17 | 5.09E-05 | 7.70E-03 |
| cell projection organization | 2.15 | 2.68E-09 | 1.85E-06 |
| plasma membrane bounded cell projection organization | 2.15 | 7.06E-09 | 3.51E-06 |
| nervous system development | 2.14 | 1.48E-18 | 2.35E-14 |
| regulation of nervous system development | 2.12 | 2.08E-07 | 6.63E-05 |
| regulation of secretion by cell | 2.12 | 1.40E-05 | 2.48E-03 |
| regulation of neurogenesis | 2.08 | 3.06E-06 | 6.84E-04 |
| cell-cell signaling | 2.07 | 5.64E-08 | 2.09E-05 |
| regulation of cellular localization | 2.03 | 8.11E-07 | 2.30E-04 |

Results displayed at FDR $p<0.05$ and Fold Enrichment>2. FDR: False Discovery Rate

**Table S5**: Gene Ontology over-representation analysis of genes associated with the ordering symptom-related cortical distant attractor connectivity map

| GO biological process | Fold Enrichment | Raw P-value | FDR |
|---|---|---|---|
| transmission of nerve impulse | 7.74 | 1.98E-05 | 1.43E-02 |
| synaptic vesicle exocytosis | 6.77 | 4.72E-05 | 2.68E-02 |
| neurotransmitter secretion | 5.16 | 4.70E-05 | 2.87E-02 |
| signal release from synapse | 5.16 | 4.70E-05 | 2.76E-02 |
| multicellular organismal signaling | 4.74 | 1.81E-05 | 1.44E-02 |
| neurotransmitter transport | 4.36 | 3.92E-05 | 2.49E-02 |
| regulation of exocytosis | 3.82 | 1.02E-05 | 1.01E-02 |
| regulation of heart contraction | 3.54 | 2.46E-05 | 1.70E-02 |
| regulation of blood circulation | 3.33 | 1.68E-05 | 1.41E-02 |
| synaptic signaling | 3.03 | 1.22E-06 | 2.78E-03 |
| chemical synaptic transmission | 2.99 | 6.28E-06 | 9.07E-03 |
| anterograde trans-synaptic signaling | 2.99 | 6.28E-06 | 8.31E-03 |
| trans-synaptic signaling | 2.98 | 4.07E-06 | 7.19E-03 |
| inorganic ion transmembrane transport | 2.86 | 6.34E-08 | 3.36E-04 |
| inorganic cation transmembrane transport | 2.75 | 1.92E-06 | 3.82E-03 |
| metal ion transport | 2.68 | 9.29E-07 | 2.95E-03 |
| modulation of chemical synaptic transmission | 2.59 | 7.73E-05 | 4.24E-02 |
| regulation of trans-synaptic signaling | 2.58 | 7.96E-05 | 4.22E-02 |
| cation transmembrane transport | 2.55 | 5.37E-06 | 8.53E-03 |
| regulation of transmembrane transport | 2.53 | 1.84E-05 | 1.39E-02 |
| regulation of vesicle-mediated transport | 2.42 | 9.27E-05 | 4.61E-02 |
| ion transmembrane transport | 2.37 | 1.15E-06 | 3.03E-03 |
| regulation of secretion | 2.20 | 8.31E-05 | 4.26E-02 |
| cation transport | 2.19 | 3.87E-05 | 2.57E-02 |
| transmembrane transport | 2.01 | 6.62E-06 | 7.51E-03 |

Results displayed at FDR $p<0.05$ and Fold Enrichment>2. FDR: False Discovery Rate

**References:**

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# Discussion

# 5. Discussion

## 5.1 Summary of key findings

The aim of this thesis was to explore the neurobiological underpinnings of subclinical obsessive-compulsive symptoms in a large sample of healthy children. Such symptoms are phenomenologically similar to OCD and their presence during childhood has been linked to the development of the disorder later in life (Fullana et al., 2009). Hence, the neuroimaging and genetic features presented herein may eventually be associated with the development of OCD and, thus, may serve to characterize high-risk individuals.

Regarding **sociodemographic and psychometric variables**, we found that the OCI-CV scores of our sample were similar, in some cases lower, to those reported in a Spanish validation sample (Rosa-Alcázar et al., 2014). Similarly, SDQ scores were lower than those of a normative population (Barriuso-Lapresa, Hernando-Arizaleta and Rajmil, 2014). Importantly, sex (i.e. girls and boys) and age (i.e. younger and older based on to the median of the age) subgroups did not differ in school performance, mother's education or OCI-CV total and symptom-specific scores. The presence of subthreshold ADHD symptoms influenced the sample of children included in each study. For instance, in our first study we reported our structural findings on the whole sample (N=255), but we performed sensitivity analyses excluding the 25 children with ADHD symptoms (n=230), which showed that our findings remained significant after excluding these cases. Conversely, in our second and third functional studies, we presented our results directly on the sample without ADHD symptoms (n=227) to avoid confounding effects.

Regarding neurobiological findings (summarized in **Table 4** at the end of this section), in **Study 1**, we aimed at assessing the GM and WM regional volumes associated with distinct dimensions of subclinical obsessive-compulsive symptoms and their putative interactions with age and sex. Importantly, we did not detect any significant associations between global symptoms and regional GM or WM volumes. In contrast, *ordering* symptoms were negatively associated with GM volumes in the bilateral ventral caudate nuclei, including the nucleus accumbens and extending to the subgenual ACC. *Obsessing*

symptoms correlated negatively with the GM and WM volumes in the right temporal pole, whereas *hoarding* symptoms were positively associated with GM and WM volumes in the left IFG. Finally, *doubting/checking* symptoms correlated positively with the WM volumes of two clusters located at the right IFOF and the isthmus of the corpus callosum. Conversely, these symptoms were negatively associated with GM volumes in the left MFG, comprising the vlPFC, albeit only at a trend level ($p<0.005$). Remarkably, none of this clusters exhibited a significant interaction with sex. However, we found an effect of age on the positive association between *hoarding* symptoms and the GM volumes in the left IFG, which was specifically more positive in children older than 10 years old. The assessment of interactions with sex and age revealed that the negative correlation between *ordering* symptoms and the GM volumes in the ventral caudate nuclei was significantly more negative in the group of boys younger than 10 years old. Nevertheless, none of the interactions with age was replicated when assessing age as a continuous variable.

In **Study 2**, we assessed the resting-state functional connectivity correlates associated with distinct subclinical obsessive-compulsive symptoms and we also evaluated their potential interactions with age and sex. We decided to complement our structural findings with the functional connectivity correlates of these symptoms because, intuitively, both subclinical and clinical manifestations of mental health disorders seem to be more related to functional rather than structural alterations. Moreover, resting-state functional connectivity assessments, in comparison to task-based fMRI, have the advantage of avoiding the task performance variability while characterizing large-scale brain intrinsic patterns of activation.

In contrast to our structural study, we observed a negative correlation between total symptoms and the global connectivity of the left ventral putamen and the left medial-dorsal thalamus, but we did not detect significant correlations between any symptom-specific dimension and global connectivity strength. In post-hoc seed-based analysis, using the left ventral putamen and the left medial-dorsal thalamus as seeds of interest, we found that total symptoms correlated negatively with the functional connectivity between these areas and a widespread bilateral pattern across the vlPFC/frontal opercula and insulae to subcortical striatal, thalamic and limbic regions, and also

including premotor and sensorimotor cortices and posterior striate and extrastriate cortices. Importantly, we also found symptom-specific correlations: *ordering* symptoms correlated negatively with the functional connectivity between the left ventral putamen and the left putamen and pallidum, as well as a sensorimotor cluster comprising the right precentral gyrus and the left Rolandic operculum and extending to the left supramarginal and superior temporal gyri. Similarly, *obsessing* symptoms also correlated inversely with the functional connectivity between the putamen seed and a limbic cluster including the right hippocampus and the left thalamus and amygdala. *Hoarding* symptoms were negatively associated with the functional connectivity between the putamen seed and the left medial-dorsal thalamus, but also with the connectivity between the left medial-dorsal thalamus seed and a widespread pattern of cortical and subcortical regions, including anterior and posterior brain regions (i.e. the left MFG, the cuneus and the calcarine sulcus) as well as the bilateral striata, including the subthalamic nucleus. Finally, *doubting/checking* symptoms correlated negatively with the connectivity between the thalamic seed and the left anterior insula. In this study, we did not detect any significant interaction between age and the above-mentioned findings. Conversely, we detected an effect of sex on the association between *hoarding* symptoms and the connectivity between the thalamic seed and the subthalamic nucleus, which was significantly more negative in girls. Similarly, assessing interactions with sex and age revealed that the relationship between *hoarding* symptoms and the connectivity between the thalamic seed and posterior cortical regions (i.e. cuneus and calcarine sulcus) was significantly more negative in the group of girls older than 10 years of age.

In **Study 3**, we assessed the presence of local and distant attractors associated with total and dimensional subclinical obsessive-compulsive symptoms and we explored their genetic signature and the functional overrepresentation of the genes expressed in symptom-related attractor areas. Then, we located the SNPs that modified gene expression in these specific areas, and we extracted their doses from our GWA data. Finally, we assessed whether the presence of minor alleles of these SNPs modulated the association between subclinical obsessive-compulsive symptoms and attractor connectivity. We found that total symptoms were negatively correlated with a local

attractor in the left ventral putamen but positively correlated with distant attractors in the left hippocampus and the bilateral SMA. The genes expressed in the hippocampal attractor contributed to multiple biological processes, among them, glutamatergic neurotransmission, fear response, and learning. Moreover, we observed a positive correlation between *ordering* symptoms and a distant attractor in the right superior parietal cortex. Genes expressed in this area contributed to different biological processes, among them, nervous impulse transmission and cellular transport. After assessing the genetic signature of these attractors, we found that, in our sample, the relationship between total symptoms and the distant attractor connectivity of the left hippocampus was only significant in children with copies of the minor allele C in the SNPs rs7613638 of the GRM7 gene, rs11145747 of the GNAQ gene and rs1994904 of the PARVA gene. Conversely, the association between *ordering* symptoms and the distant attractor connectivity of the right superior parietal cortex was only significant in children with copies of the minor allele T in the SNP rs2143290 of the ATP1B1 gene, and in those with no copies of the minor allele C in the SNP rs6490097 of the TESC gene.

**Table 4**. Summary of the neurobiological findings from the three studies included in this thesis

| | **Ordering OCI-CV** | **Obsessing OCI-CV** | **Hoarding OCI-CV** | **Doubting/Checking OCI-CV** | **Total OCI-CV** |
|---|---|---|---|---|---|
| **STUDY 1: VBM** | ↓ GM v caudate R/L[1]<br>[1]boys <10 yo | ↓ GM & WM temporal pole R | ↑ GM[2] & WM IFG L<br>[2]children >10 yo | ↑ WM IFOF R & CC | – |
| **STUDY 2: Global FC** | – | – | – | – | ↓ Global FC v putamen L & md thalamus L |
| **STUDY 2: Seed-based FC** Seed: v putamen L | ↓ FC putamen L, rolandic operculum / superior temporal gyrus L, supramarginal gyrus L, precentral gyrus R | ↓ FC hippocampus R, thalamus L, amygdala L | ↓ FC md thalamus L | – | ↓ FC widespread bilateral pattern of regions ^ |
| **STUDY 2: Seed-based FC** Seed: md thalamus L | – | – | ↓ FC putamen R/L, STN R[3], md thalamus L, cuneus-calcarine R/L[4], MFG L<br>[3]girls; [4]girls >10 yo | ↓ FC insula L | ↓ FC widespread bilateral pattern of regions ^ |
| **STUDY 3: Attractor FC** | ↑ Distant attractor FC superior parietal R | – | – | – | ↓ Local attractor FC v putamen L<br>↑ Distant attractor FC hippocampus L & SMA R/L |
| **STUDY 3: SNPs that moderate attractor FC-symptom associations** | rs2143290 (ATP1B1)<br>rs6490097 (TESC) | – | – | – | rs7613638 (GRM7)<br>rs11145747 (GNAQ)<br>rs1994904 (PARVA) |

Superscript numbers highlight the findings that were more prominent in a specific sex and/or age group; ^: widespread bilateral pattern across the vlPFC/frontal opercula and insulae to subcortical striatal, thalamic and limbic regions, and also including premotor and sensorimotor cortices and posterior striate and extrastriate cortices; CC: Corpus Callosum; FC: Functional Connectivity; GM: Grey Matter; IFG: Inferior Frontal Gyrus; IFOF: Inferior Fronto-Occipital Fasciculus; L: Left; md: medial-dorsal; MFG: Middle Frontal Gyrus; OCI-CV: Obsessive-Compulsive Inventory-Child Version; R: Right; v: ventral; SMA: Supplementary Motor Area; SNPs: Single-Nucleotide Polymorphisms; STN: SubThalamic Nucleus; WM: White Matter; yo: years old.

## 5.2 Interpretation of key findings

To our knowledge, no previous studies have used a dimensional approach to assess the neurobiological correlates of subclinical obsessive-compulsive symptoms neither in adults nor in youth. Hence, the interpretation of our findings is limited to the comparison with clinical samples. In this section, we will first highlight the general differences that we observed between assessing symptoms from a global and a symptom-specific perspective in our structural and functional findings. Then, we will deepen into the interpretation of the findings observed across the three studies presented in this thesis, grouping them into symptom dimensions (i.e. *ordering, obsessing, hoarding* and *doubting/checking*) and total symptoms.

Remarkably, we did not find structural changes associated with total obsessive-compulsive symptoms (**Study 1**). In contrast, when assessing functional connectivity, our main findings were related to total symptoms (**Studies 2 and 3**). This could be explained by the fact that both clinical and subclinical manifestations of OCD may be more closely linked to functional rather than structural changes. This idea is supported by the current neurobiological models of OCD, which have been developed based on brain functional data and, more precisely, on functional connectivity (Menzies et al., 2008; Soriano-Mas and Harrison, 2017). Nevertheless, when assessing symptoms as dimensional constructs, we found distinct structural correlates for *doubting/checking, hoarding, obsessing* and *ordering* dimensions (**Study 1**) but no global functional connectivity correlates (**Study 2**). However, when implementing a seed-based approach using the regions associated with total symptoms (i.e. left ventral putamen and left medial-dorsal thalamus) as seeds of interest, again, we observed distinct patterns of connectivity alterations for each dimension (**Study 2**), which suggests a common alteration in relay nodes of the CSTC circuit across dimensions but also symptom-specific alterations involving distinct circuits. Additionally, in **Study 3**, the *ordering* dimension was the only dimension that correlated with the attractor connectivity of a certain brain node. Taken together, our findings show the importance of assessing both total and symptom-specific symptoms to identify not only the shared alterations but also the neurobiological differences across symptom dimensions.

***Ordering symptoms***

Focusing on symptom-specific findings, the *ordering* dimension was associated with decreased regional GM volume in the bilateral ventral caudate and with decreased functional connectivity between the left ventral putamen and sensorimotor dorsal striatum and sensorimotor cortices. Moreover, *ordering* symptoms positively correlated with the distant attractor connectivity of the right superior parietal cortex.

The ventral caudate is an important relay station of the ventral cognitive CSTC loop, which mediates response inhibition (Simpson et al., 2020). Hence, changes in the GM volume of this are may be indicative of alterations in the ventral cognitive loop and its functions. In this line, previous research in clinical samples has shown that the inability to activate the ventral caudate was linked to cognitive flexibility impairments in youth (Britton et al., 2010) and that deep-brain stimulation of this area improved OCD symptom severity in treatment-resistant adults (Alonso et al., 2015). Focusing on previous structural research, a large body of evidence supports the notion of increased ventral striatal volumes in individuals with OCD. However, as mentioned in the introduction, this finding involves predominantly the ventral putamen and has been associated with increasing age (Pujol et al., 2004; Radua and Mataix-Cols, 2009; de Wit et al., 2014; Picó-Pérez et al., 2020). In contrast, our finding was more prominent in boys younger than 10 years of age. From a neurodevelopmental perspective, it may be interpreted as an alteration of the normal volumetric increases observed in subcortical regions during childhood (Fareri, Martin and Delgado, 2008). It could also be linked to the slower developmental rate of the ventral caudate that boys exhibit in comparison to girls at these ages (Giedd et al., 1996), which could explain the higher incidence of early-onset OCD in boys (Ruscio et al., 2010). This idea is congruent with the earlier onset of *ordering* symptoms and with the higher prevalence of *ordering* symptoms in early-onset OCD, compared to other OCD dimensions (Uguz et al., 2006; Labad et al., 2008; Nakatani et al., 2011; Kichuk et al., 2013). Nevertheless, we did not assess the pubertal status of participants and the age effect was not observed when assessing age as a quantitative variable, hence, caution is warranted when interpreting this effect. A possible explanation for the lack of a quantitative association could be that

developmental effects do not accumulate in a progressive manner through years, but across different neurodevelopmental stages.

While our structural findings preliminarily suggest that *ordering* symptoms may be exclusively mediated by the ventral cognitive CSTC loop, our functional studies add another variable to the equation, since the ventral putamen showed decreased functional connectivity with regions of the sensorimotor CSTC loop, mediating stimulus-response, habit-related, behaviors (Simpson et al., 2020). Moreover, we also observed increased distant attractor connectivity in the superior parietal cortex, which is a multimodal brain hub involved in visuospatial and attentional processing and in executive functioning (van den Heuvel and Sporns, 2011; 2013). Since total connectivity assessments tend to overestimate local or modular connectivity (i.e. connectivity linking nodes at 1-step distance of the topological space), our finding assessing total connectivity could be interpreted as individuals with *ordering* symptoms exhibiting impaired modular connectivity within the sensorimotor CSTC loop, which could alter the capacity to disengage from particular motor patterns, resulting in repeated sequences of movements or actions. On the other hand, increased distant attractor properties of the superior parietal hub may lead dynamic connectivity, possibly from distant primary sensory areas, to converge and reverberate in this area. This could lead to the brain getting locked in specific overstable cognitive states that impair the correct and flexible functioning of visuospatial and attentional processing and executive functions, which could alter the ability to represent and manipulate objects, therefore leading to the manifestation of *ordering* symptoms. Notably, these functional findings concur with previous research associating *ordering* symptoms with structural alterations in the motor and parietal cortex of adults with OCD (van den Heuvel et al., 2009), and linking sensory phenomena, which is more prevalent in individuals with OCD that exhibit *ordering* symptoms, to structural sensorimotor alterations (Subirà et al., 2015). Likewise, they are consistent with the well-known link between this dimension and motor tic disorders (van den Heuvel et al., 2009). From a neuropsychological perspective, our interpretation is in line with a recent meta-analysis in adults with OCD reporting that the *ordering* dimension was linked to overall worse neuropsychological performance, and, specifically, in the attention, visuospatial ability, and verbal working memory domains,

compared to the *obsessing/checking* dimension (Bragdon, Gibb and Coles, 2018). Similarly, in youth with OCD, *ordering* symptoms have been associated with greater cognitive impairment with specific deficits in nonverbal fluency, processing speed and response inhibition and switching (McGuire et al., 2014). Taken together, our structural and functional findings indicate that the alterations linked to the *ordering* dimensions are related to cortical and subcortical nodes of the sensorimotor CSTC loop, a subcortical node of the ventral cognitive CSTC loop (specifically in younger boys), and a key cortical hub outside the CSTC circuit.

Importantly, we found that the presence of the minor allele T in the SNP rs2143290 of the ATP1B1 gene acted as a risk factor for the *ordering* symptom-related right superior parietal attractor, whereas the presence of the minor allele C in the SNP rs6490097 of the TESC gene acted as a protective factor. These genes encode the β1 subunit of the Na+/K+ ATPase (NKA) and the tescalcin, which mediates the Na+/H+ exchanger 1 (NHE1) activity, respectively. Hence, they contribute to balance the electrochemical gradients at the plasma membrane. This function is key for electrical excitability and synaptic transmission and, thus, to glutamatergic neurotransmission. For instance, NKA is involved in astrocytic glutamate uptake and seems to interact with NMDA glutamate receptors (Illarionova et al., 2014; de Lores Arnaiz and Bersier, 2014), whereas inhibition of NHE1 activity protects from NMDA receptors-induced excitotoxicity (Lam et al., 2013). Hence, it is possible that polymorphisms in ATP1B1 and TESC modify their expression and, thus, alter the activity of NKA and NHE1, leading to either increased or reduced risk for overactive glutamatergic neurotransmission, which is known to heighten attractor properties. Since these genes are also expressed in other brain areas, these molecular-level alterations should be expected to be not specifically related to *ordering* symptoms, although, according to our findings, when they are observed in the superior parietal cortex area they seem to be linked to *ordering* symptoms.

***Obsessing symptoms***

The *obsessing* dimension correlated with decreased regional GM and WM volumes in the right temporal pole and with decreased functional connectivity between the left

ventral putamen and limbic regions (i.e. right hippocampus and left thalamus and amygdala). Form a functional perspective, the limbic system mediates anxiety, emotional processing and extinction learning. Similarly, the temporal pole has been considered part of the extended limbic system because it is connected to limbic and paralimbic regions and is involved in emotional and social functioning and, more specifically, in moral cognition (Zahn et al., 2009). Therefore, our findings coincide in that *obsessing* symptoms are related to alterations in limbic areas and, thus, to impaired emotion and anxiety processing and moral cognition. Remarkably, our structural finding is in line with previous structural studies linking decreased GM volume in the temporal pole with autogenous obsessions (i.e. that emerge without the trigger of an identifiable external stimuli) (Subirà et al., 2013), and with OCD-related dysfunctional beliefs in healthy subjects (Alonso et al., 2013). Likewise, our functional connectivity findings concur with a previous study reporting that *aggressive* symptoms, which in our assessment scheme would be categorized as *obsessing* symptoms, predicted diminished functional connectivity between the ventral striatum and the amygdala (Harrison et al., 2013). Therefore, such connectivity impairments may account for the heightened fear and threat estimation processes, which, alongside increased amygdala activation during emotional processing (Via et al., 2014), seem to be a distinctive feature of the *aggressive* or *sexual/religious* symptomatology. Additionally, a functional study linked the activation of the temporal pole and limbic and paralimbic areas with the increased stress response observed in individuals with OCD during the presentation of moral dilemmas (Harrison et al., 2012). Hence, dysfunctional beliefs, such as increased importance and control of thoughts and feelings of guilt, which have a moral component (Shapiro and Stewart, 2011) and have been associated with *unacceptable/taboo thoughts* (Wheaton et al., 2010; Brakoulias et al., 2014), may be mediated by either structural and/or functional impairments in these areas.

***Hoarding symptoms***

In our studies, the *hoarding* dimension positively correlated with increased regional GM and WM volumes in the left IFG. At the functional level, *hoarding* was the only dimension associated with decreased functional connectivity between the two seeds of interest

(i.e. left ventral putamen and dorsal-medial thalamus) and between the left dorsal-medial thalamus and a widespread pattern of subcortical and cortical regions. Importantly, both our structural and functional findings interacted with age and sex. Unfortunately, the interpretation of these findings is hindered by the limited literature on the neurobiological underpinnings of hoarding symptoms in pediatric samples and by the scarce representation of patients with these symptoms in general OCD samples.

From a functional perspective, the IFG and the ventral putamen are nodes of the ventral cognitive CSTC circuit, which, as mentioned earlier, is involved in response inhibition and cognitive control of emotion. Hence, our findings are suggestive of an impairment in this circuit in individuals with *hoarding* symptoms. In agreement, a previous study observed decreased connectivity between the thalamus and the striatum (Levy et al., 2019), albeit this finding involved the caudate and we did not observe significant findings in this region. Likewise, task-based fMRI studies have shown that individuals with OCD exhibited abnormal activations of the IFG during cognitive control and conflict processing (Roth et al., 2007; Tops and Boksem, 2011), and *hoarding* symptoms have been consistently linked to impaired response inhibition and switching (Morein-Zamir et al., 2014; Suñol et al., 2019). In contrast, our structural finding contradicts previous research describing decreased GM volumes in this region associated with OCD (de Wit et al., 2014), but this inconsistency could stem from the above-mentioned limitation of underrepresentation of individuals with *hoarding* symptoms in OCD samples.

Nevertheless, *hoarding* symptoms also correlated negatively with the functional connectivity between the thalamic seed and a large pattern of subcortical and cortical regions including the subthalamic nucleus, the putamen, the medial dorsal thalamus, the dlPFC and medial occipital cortices. The involvement of the dlPFC suggests that *hoarding* symptoms may also be linked to functional connectivity impairments in the dorsal cognitive loop and suggests that these symptoms may be associated with a more pervasive alteration of multiple CSTC loops. Moreover, it concurs with previous research reporting executive function deficits and abnormal activation of this region during response inhibition, set-shifting and error processing in individuals with hoarding disorder (Levy et al., 2019; Suñol et al., 2019). Conversely, the negative correlation between the *hoarding* dimension and the connectivity between the thalamic seed and

medial occipital regions is difficult to link to specific psychological processes prompting *hoarding* symptoms, but it is suggestive of the involvement of areas outside this circuit.

From a neurodevelopmental perspective, the increased GM volume of the IFG associated to these symptoms was specific of participants older than 10 years old. In normal neurodevelopment, synaptic pruning triggers a decrease in the GM volume of frontal lobes after the age of 10. Hence, *hoarding*-related GM increases may reflect neurodevelopmental disruptions. Additionally, the association between the symptoms and the connectivity between the thalamic seed and the subthalamic nucleus was more prominent in girls, whereas the association between the symptoms and the connectivity between the thalamic seed and the medial occipital regions was specific of girls older than 10 years old. Taken together, our findings suggest that pre-adolescent girls may exhibit increased vulnerability to *hoarding* symptoms. Notably, previous studies have shown that women with *hoarding* behavior present more severe obsessive-compulsive symptoms (Samuels et al., 2008), and that these symptoms are more strongly familial than other OCD dimensions, correlating strongly in mother-daughter dyads and in female-female sibling pairs (Hasler et al., 2007; Samuels et al., 2008; Taberner et al., 2009). Interestingly, the contribution of genetic and environmental factors on *hoarding* symptoms seems to change throughout the years and, possibly, in interaction with sex. In this line, a twin study revealed that environmental effects contribute to *hoarding* symptoms in 15-year-old girls, but not in other age or sex groups (Ivanov et al., 2017). Parental style seems to be a relevant environmental factor because women with OCD and *hoarding* symptoms report low maternal care and high maternal overprotection, compared to the non-hoarding group. In fact, researchers found that the low care/high overprotection group had significantly greater odds of exhibiting *hoarding* symptoms than the high care/low overprotection group (Chen et al., 2017). Our finding is likely to be the first neurobiological correlate of the link between *hoarding* symptoms and female sex during adolescence that has been observed in the clinical and epidemiological contexts. However, as stated for the *ordering* results, we did not assess the pubertal status of the participants, hence, caution is warranted.

Taking into account that only a few studies have assessed the brain correlates of *hoarding* symptoms, further research is needed to determine the extent of alterations

in subjects with these symptoms and the putative differences between individuals with OCD and *hoarding* symptoms, even at subclinical stages, and individuals with hoarding disorder. Similarly, the modulatory effects of sex and age in relation to these symptoms deserve further attention.

***Doubting/Checking symptoms***

The *doubting/checking* dimension positively correlated with the WM volumes at the right IFOF and the isthmus of the corpus callosum and, at a trend level, negatively with the GM volumes in the left MFG, comprising the vlPFC. Moreover, these symptoms were also negatively associated with the functional connectivity between the left medial-dorsal thalamus and the left anterior insula.

In our structural *hoarding*- and *obsessing*-related findings, WM changes overlapped with GM alterations, which may reflect that the volumetric changes extended to the afferent and efferent projections of these regions. In contrast, in the case of *doubting/checking* the WM findings did not overlap with the GM one but, instead, involved the IFOF and the callosal isthmus. The IFOF tract links the ventrolateral and medial OFC to posterior parietal and occipital cortices, whereas the callosal isthmus has fibers connecting the superior temporal and inferior parietal areas (Eccher, 2014). Notably, in normal development, WM volume increases steadily until adulthood (Gerber et al., 2009), thus, these findings may reflect accelerated WM maturation in association with *doubting/checking* symptoms. More specifically, alterations in the IFOF may contribute to impaired prefrontal functioning (Wu et al., 2016), and changes in the callosal isthmus may reflect protracted maturation of this section of the corpus callosum in comparison to more anterior regions in which fibers transfer information between frontal lobes (Luders, Thompson and Toga, 2010; Eccher, 2014). In agreement, previous studies have shown that, among other regions, the IFOF and the corpus callosum exhibited increased FA in youth with OCD (Zarei et al., 2011; Gruner et al., 2012; Fitzgerald et al., 2014; Koch et al., 2014) but decreased FA in adults with the disorder (Garibotto et al., 2010; Koch et al., 2014; Gan et al., 2017). Alteration of frontal processing and also the involvement of the ventral cognitive CSTC loop is supported by the negative association between

*doubting/checking* and GM volumes in the vlPFC. This concurs with previous research in OCD samples reporting decreased volumes (Piras et al., 2015) and connectivity impairments in this area accounting for reduced cognitive flexibility (Vaghi et al., 2017), which has been associated with *checking* in both clinical and healthy samples (Leopold and Backenstrass, 2015; Snorrason et al., 2016; Cameron et al., 2020). Similarly, the decreased functional connectivity between the thalamic seed and the anterior insula that we found to be associated with *doubting/checking* symptoms could putatively involve the ventral cognitive circuit and, therefore, contribute to cognitive flexibility impairments.

Nevertheless, the thalamus and the anterior insula are key components of the salience network (SN). Hence, functional connectivity alterations between these areas may impede the correct processing of salient stimuli in uncertain environments (Song et al., 2011), which may lead to an abnormally heightened emotional salience of stimuli. Hypothetically, *checking* compulsions may arise as a coping mechanism to reduce the increased anxiety levels caused by the altered functioning of the SN. Consistent with this interpretation, reduced connectivity between these regions has been linked to increased symptom severity in adults with OCD (Stern et al., 2012). More precisely, when compared to OCD non-checkers and healthy controls, OCD checkers exhibited heightened intolerance of uncertainty (Tolin et al., 2003; Simmons et al., 2008), which has been associated with increased activation of the insula (Simmons et al., 2008). Likewise, volumetric alterations in the insula seem to be more prominent in individuals with *checking* as their predominant OCD symptom dimension (Song et al., 2011).

### *Total Obsessive-Compulsive Symptoms*

Finally, total obsessive-compulsive symptoms negatively correlated with the global functional connectivity of the left medial-dorsal thalamus and the left ventral putamen. Similarly, these symptoms correlated with decreased local attractor properties in the left ventral putamen, but also with increased distant attractor properties in the SMA and the left hippocampus.

From a functional perspective, the medial-dorsal thalamus is the final relay station of distinct CSTC circuits, hence, we could not determine if decreased global connectivity in this area may be suggestive of an alteration in a specific CSTC loop or of a more pervasive alteration involving distinct CSTC circuits. Nevertheless, our finding concurs with previous meta- and mega-analyses reporting volumetric abnormalities in the thalamus, especially in youth with OCD (Rotge et al., 2009; Boedhoe et al., 2017).

In contrast, the ventral putamen connects the vlPFC with the thalamus and, thus, acts as the principal relay station of the ventral cognitive CSTC loop. Hence, the negative association that we observed between total symptoms and the global connectivity of this area may reflect a diminished crosstalk within the ventral cognitive loop in children with obsessive-compulsive symptoms. This concurs with the fact that, across studies, we found ventral cognitive loop abnormalities to be associated with distinct symptom dimensions. Also, it is in agreement with previous research showing a negative association between the resting-state activity of the ventral putamen and the Y-BOCS compulsions subscore in adults with OCD (Giménez et al., 2017) and, more specifically, with studies reporting reduced functional connectivity within the ventral cognitive loop both in youth and adults with OCD (Harrison et al., 2009; Bernstein et al., 2016). Remarkably, the negative association between the total obsessive-compulsive symptoms and the functional connectivity of the left ventral putamen was replicated in our third study, where, interestingly, symptoms correlated exclusively with the local attractor properties of this area. This supports the notion that, due to the increased connectivity strength observed between neighbor nodes, assessing functional connectivity in a global, averaged manner may highlight local connectivity while overshadowing distant patterns of connectivity. Hence, it emphasizes the importance of segregating functional connectivity to also account for distant connectivity features.

The concept of a negative total symptom-related local attractor in the left ventral putamen may be interpreted as this region of the ventral cognitive loop losing its ability to attract connectivity coming from neighboring nodes. Importantly, since we segregated connectivity based on modularity, these neighbor nodes do not have to be physically close to the ventral putamen. However, our finding contradicts a previous study reporting decreased local connectivity in this area in medicated compared to

unmedicated OCD patients and associating increased distant connectivity in this area with OCD severity (Beucke et al., 2013). Since they segregated connectivity based on Euclidean distances, their findings indicate increased modular connectivity in this area, which is the opposite of what we found. Such differences could reflect distinct connectivity impairments between subclinical and clinical obsessive-compulsive symptoms or between pediatric and adult samples with these symptoms, albeit they could also only reflect sample size and methodological differences. Further research is warranted to elucidate this issue.

In contrast, the SMA is part of the sensorimotor CSTC loop that, as mentioned earlier, mediates stimulus-response based habitual behavior (Simpson et al., 2020). Importantly, this region receives motor inputs from distinct subcortical regions through the glutamatergic projections of the thalamus (DeLong and Wichmann, 2010) and contributes to planning, initiation, inhibition, maintenance and repetition of action (Nachev, Kennard and Husian, 2008). Therefore, the increased distant attractor properties in the SMA that we found to be associated with total obsessive-compulsive symptoms may cause the distant subcortical connectivity outputs to converge and reverberate in this area. Plausibly, this could impair the sensorimotor CSTC loop and obstruct the capacity of disengaging from certain motor patterns, thus facilitating the repetition of specific sequences of movements or actions. Such interpretation concurs with meta-analyses describing the efficacy of inhibitory low-frequency repetitive transcranial magnetic stimulation (LF-rTMS) targeting the SMA on improving OCD symptom severity (Berlim, Neufeld and Van den Eynde, 2013; Zhou et al., 2017). The fact that in our second study we observed decreased total connectivity between the ventral putamen and areas of the sensorimotor CSTC loop in association with *ordering* symptoms does not necessarily contradict this current finding. Possibly, increased attractor properties in the SMA could hijack distant functional connectivity and, thus, contribute to deficits of modular connectivity within areas of the sensorimotor loop and also in the inter-connectivity between ventral cognitive and sensorimotor areas of the CSTC circuit, which could predispose to *ordering* symptoms. However, additional research is warranted to confirm this hypothesis.

Finally, regarding hippocampal findings, this is a limbic region that receives multiple inputs from association and prefrontal cortices via the entorhinal cortex, mediating memory processing, integration spatial and affective information, and processing of approach/avoidance conflict (Battaglia et al., 2011; Ito and Lee, 2016). The heightened distant attractor properties of this area that we found to be associated with total obsessive-compulsive symptoms could lead to distant cortical connectivity to converge and reverberate in the hippocampus, then contributing to abnormal processing and integration of negative stimuli. This interpretation is in line with neuropsychological studies showing that individuals with OCD exhibit a memory bias towards thread-related stimuli (Muller and Roberts, 2005) and a biased avoidance tendency (Endrass et al., 2011). Although our finding concurs with a study reporting that increased glucose metabolism in the hippocampus was associated with OCD symptom severity (Kwon et al., 2003) and with a recent meta-analysis showing increased GM volumes in the hippocampus independent of medication status (Picó-Pérez et al., 2020), opposite findings have been also observed. Likewise, functional connectivity findings have been heterogeneous, possibly due to small sample sizes and different methodological approaches. Nevertheless, a previous graph-theory based study reported that, compared to controls, individuals with OCD exhibited decreased inward connectivity (i.e. proxy of local connectivity) but not outward connectivity (i.e. proxy of distant connectivity) in the hippocampus, albeit reduced outward connectivity was observed in the amygdala and the parahippocampal region (Göttlich et al., 2014). It is therefore possible that obsessive-compulsive symptoms are related to decreased local connectivity but increased distant connectivity in this region. This would explain why we observed decreased total connectivity (which is known to hinder distant connectivity) between the ventral putamen and the hippocampus in association with *obsessing* symptoms. Further research is needed to confirm this phenomenon in OCD samples.

Additionally, as hypothesized by Rolls, Loh and Deco (2008), we found that increased attractor properties of the hippocampus were associated with glutamatergic neurotransmission. On the one hand, we observed that genes expressed in this region were functionally related to biological processes involved in glutamatergic transmission and, on the other hand, we detected that the presence of the minor allele C in the SNP

rs7613638 of GRM7 and in the SNP rs11145747 of GNAQ glutamate-related genes predisposed to the emergence symptom-related attractor properties in the hippocampus. The GRM7 gene encodes the metabotropic glutamate receptor 7 (mGluR7), which is part of the G protein-coupled group III of metabotropic glutamate receptors that regulate the function of NMDA receptors (Fisher et al., 2018), and modulates excitatory transmission in the hippocampus (Cosgrove et al., 2011). The GNAQ gene encodes the Gαq, which mediates intracellular signaling pathways through the activity of Gαq-protein-coupled receptors (GPCRs), such as the group I metabotropic glutamate receptors (i.e. mGluR1 and mGluR5), and also contributes to regulate the activation of NMDA receptors in the hippocampus (MacDonald, Jackson and Beazely, 2007). Consequently, we hypothesize that polymorphisms in these genes increase glutamatergic synaptic transmission in the hippocampus, which may alter its plasticity and attractor properties contributing to negative memory and aversive propensity biases. Such interpretation is in agreement with preclinical research reporting that mGluR7 knockdown in the hippocampus and basolateral amygdala leads to impairments in extinction of learned aversion (Fendt et al., 2008), whereas Gαq depletion in glutamatergic cells within these areas caused spatial memory deficits (Graham et al., 2015). A similar risk factor effect was observed in the presence of the minor allele C in the SNP rs1994904 of the PARVA gene, which encodes the α-parvin, an actin-binding protein involved in cell adhesion, motility and survival. Albeit it is not directly linked to glutamatergic transmission or with OCD, a study showed that the regulation of actin cytoskeleton, cell adhesion molecules and actin binding were biological processes associated to increased risk for OCD (Yue et al., 2016). Thus, the association between PARVA gene and symptom-related attractor properties in the hippocampus may be mediated by these pathways.

## 5.3 Limitations

The results presented in this thesis should be cautiously interpreted due to some methodological limitations. The specific limitations of each study are detailed in the

corresponding publications; nevertheless, there are general limitations shared across studies that deserve special consideration and that will be summarized as follows.

The main limitation is the cross-sectional nature of the studies. Since subclinical obsessive-compulsive symptoms in children have been linked to an increased risk of meeting OCD criteria later in life (Fullana et al., 2009), we tentatively considered the neurobiological features described in our studies as potential neuroimaging biomarkers of increased risk for OCD. However, only longitudinal studies will allow to discern whether our findings are predictive markers for OCD development or exclusively associated with subclinical obsessive-compulsive symptoms. Likewise, since all our studies were correlational, we cannot directly infer causality from our findings. Hence, the preliminary associations that we observed between neuroimaging, genetic and psychometric features should be interpreted with caution.

Another important limitation is that, since the recruitment of participants occurred within the framework of the BREATHE project, our studies lack a comparison group of age- and sex-matched children with an OCD diagnosis. Comparing these two groups would have allowed to determine whether the neurobiological features identified in children with subclinical obsessive-compulsive symptoms were also present in a pediatric clinical sample.

Recruiting only healthy children as participants had the advantage of avoiding the most prevalent confounders observed when assessing clinical samples, such as treatment effects, the presence of different comorbidities, or the clinical and neurobiological features associated with the course of the disorder. Nevertheless, there are worth-mentioning confounders in our sample that were not completely controlled for and, consequently, could have added variability to our neuroimaging and genetic findings. For instance, each participant underwent only one clinical interview with either a psychiatrist or a psychologist of the research group. Hence, we did not collect intra- or inter-rater reliability measurements, which inevitably limits the validity of inclusion criteria. Furthermore, we did not assess their pubertal status, their familiar history of mental health or neurological conditions, or the use of prescribed or over-the-counter medications across families.

Finally, despite we implemented different neuroimaging techniques and combined multi-source neurobiological information, the assessment of other neuroimaging sequences (e.g. DTI, task-based fMRI), as well as the implementation of other analysis techniques (e.g. surface-based or cortical thickness) would complement our findings and further advance our understanding of the neurobiological bases of subclinical obsessive-compulsive symptoms.

## 5.4 Implications for future research

In this thesis, we have attempted to assess subclinical obsessive-compulsive symptoms in healthy children from an integrative perspective, implementing different neuroimaging modalities and combining neuroimaging and genetic data. Considering the scarce literature on this topic, the three studies presented in this thesis constitute a step forward towards a better understanding of the neurobiological bases of subclinical obsessive-compulsive symptoms.

To conclude this thesis, we will discuss potential future research lines that, in our opinion, could significantly contribute to unravel the complex link between subclinical obsessive-compulsive symptoms and OCD. Firstly, most of the research tackling this issue, including the studies presented herein, has been cross-sectional. Hence, longitudinal studies with robust samples and proper follow-up clinical assessments are warranted to determine whether the neurobiological features associated with subclinical obsessive-compulsive symptoms are appropriate predictors for the future OCD development. Additionally, it would be beneficial to also include follow-up neuroimaging evaluations as a means to infer causality between brain and clinical changes.

Secondly, stemming from our own experience, we recommend the use of multidimensional approaches to assess the neurobiological correlates of the different obsessive-compulsive symptoms. To date, both structural and functional neuroimaging studies implementing such approaches have provided relatively heterogeneous findings. Nevertheless, there is a large body of evidence supporting the notion that,

although some neurobiological features may be shared across distinct symptom dimensions, there are also distinct alterations linked to different types of symptoms. This is of special relevance in longitudinal studies aimed at providing neurobiological markers of OCD development, because their findings could go a long way towards the identification of at-risk individuals and could be very informative to design prevention strategies. However, if they assess obsessive-compulsive symptoms as a whole, they run the risk of finding markers that are only associated with the most well-represented symptoms in OCD samples (i.e. doubting/checking or washing), while failing to identify the features that predispose to less prevalent symptom dimensions. Consequently, potential prevention strategies based on such findings may not be effective for a large number of individuals with OCD. In contrast, multidimensional approaches may help to identify more specific neurobiological markers and, thus, serve to design more tailored prevention strategies that suit individuals with distinct symptom profiles.

Thirdly, when assessing youth with obsessive-compulsive symptoms, it would be relevant to also take into account developmental factors such as age, sex, pubertal status or the presence of symptoms of other developmental disorders, such as ADHD. Similar to the multidimensional model, such an assessment would contribute to better characterize individuals with these symptoms and, hence, may contribute to more personalized neurobiologically-informed prevention strategies.

Fourthly, we consider that the study of both subclinical and clinical obsessive-compulsive symptoms would largely benefit from the introduction of innovative methodological approaches that allow to assess brain connectivity in a more comprehensive manner. In this line, we would like to highlight the potential of dynamic functional connectivity analyses that do not assume the staticity of functional brain networks and allow to characterize connectivity fluctuations over time. Similarly, psychiatric disorders have generally been linked to large-scale network alterations involving distant and interconnected brain areas, which has led to the need of studying psychiatric conditions from a network-based perspective (Fornito and Harrison, 2012). In this context, the role of graph theory-based methods (e.g. degree connectivity), which allow assessing alterations in network integrity and hub topography, is crucial (Bullmore and Sporns, 2009). Moreover, the fact that our ventral putamen connectivity

finding was replicated when assessing both total and local connectivity supports the notion that neighboring nodes engage with high connectivity strength and, thus, that local connectivity (i.e. between neighboring regions) may overshadow distant connectivity in total connectivity assessments (Diez and Sepulcre, 2018). Hence, we also recommend segregating local and distant connectivity in future studies.

# Conclusions

# 6. Conclusions

We draw the following conclusions from the studies included in this thesis:

- Total subclinical obsessive-compulsive symptoms are linked to CSTC circuit alterations, which concurs with prevailing neurobiological models of OCD. Nevertheless, our findings also support the involvement of regions outside this circuit in the development of these symptoms.

- Subclinical obsessive-compulsive symptom dimensions are linked to distinct neurobiological alterations. According to our findings, *obsessing* symptoms are related to limbic alterations. *Doubting/checking* symptoms are linked to changes in the ventral cognitive CSTC loop, the insula, the IFOF and the isthmus of the corpus callosum. In contrast, *ordering* symptoms are associated with changes in the sensorimotor and ventral cognitive CSTC loops and in the superior parietal cortex, while *hoarding* symptoms are related to variations in distinct CSTC loops, suggesting a more pervasive CSTC alteration.

- *Ordering*-related structural changes are specific of boys younger than 10 years of age, which suggests that this demographic group may be more vulnerable to develop *ordering* symptoms. In contrast, *hoarding*-related structural and functional changes are specific of children older than 10 years of age, especially in the female sex. Hence, preadolescent girls may show an increased susceptibility to *hoarding* symptoms.

- Polymorphisms in the GRM7 and GNAQ genes, involved in modulating glutamatergic neurotransmission, and in the PARVA gene, associated with actin-cytoskeleton, cell adhesion, motility and survival, predispose to heightened attractor properties in the hippocampus, which are linked to total obsessive-compulsive symptoms.

- Polymorphisms in ATP1B1 and TESC genes, involved in the maintenance of electrochemical gradients and regulation of cellular pH, act as a risk and a protective factor, respectively, for increased attractor properties in the superior parietal cortex, which are associated with *ordering* symptoms.

# Summary in Catalan

# 7. Summary in Catalan
# Resum en Català

## 1. INTRODUCCIÓ

### 1.1. Epidemiologia i característiques clíniques del Trastorn Obsessiu-Compulsiu (TOC)

El trastorn obsessiu-compulsiu (TOC) és un trastorn psiquiàtric caracteritzat per la presència de pensaments intrusius que causen ansietat (obsessions) i que condueixen a realitzar comportaments repetitius i ritualístics per tal de reduir-la (compulsions) (American Psychiatric Association, 2013). Aquest trastorn afecta entre l'1 i el 3% de la població (Mathes, Morabito i Schmidt, 2019) i té un profund impacte psicosocial i econòmic (Murray et al., 1996; World Health Organization, 2017). L'edat d'inici del TOC segueix una distribució bimodal amb un primer pic en la preadolescència (10 anys) i un segon pic en l'inici de l'edat adulta (19-20 anys). A més, les corbes d'edat d'inici difereixen entre sexes: els homes constitueixen la majoria de casos primerencs i les dones tenen una major incidència de casos durant l'adolescència (Geller, 2006; Ruscio et al., 2010).

### 1.2. Etiologia del TOC

Actualment no es disposa d'un model teòric capaç d'explicar la complexa etiologia del TOC. Malgrat això, en les darreres dècades s'han desenvolupat diversos models a partir de l'estudi de la neuropsicologia i la neurobiologia del trastorn:

Base neuropsicològica del TOC: La teoria metacognitiva descriu que els individus amb TOC experimenten dificultats en la metacognició que els duen a sobreestimar la importància i credibilitat dels pensaments intrusius. En conseqüència, les compulsions apareixen amb l'objectiu d'eliminar aquests pensaments i les seves possibles conseqüències negatives. Però, tot i que produeixen un alleugeriment a curt termini, a

la llarga acaben reforçant tant les compulsions com les creences desadaptatives sobre les obsessions. Aquesta ha estat la teoria que més suport ha rebut, inclòs per part d'estudis computacionals i de neuroimatge (Gillan et al., 2014; Voon et al., 2014; Guillan et al., 2015).

Base neurobiològica del TOC: Estudis de neuroimatge amb ressonància magnètica (RM) i, específicament, de connectivitat funcional han contribuït a la formulació del model cortico-estriatal-talàmic-cortical (CSTC, per les sigles en anglès) com a substrat neuroanatòmic del TOC. En aquest model es descriu que la simptomatologia del TOC es podria explicar per la hiperactivació de la via directa del circuit CSTC que connecta regions corticals frontals amb els ganglis basals i el tàlem, fet que conduiria a una selecció patològica de seqüències de comportament prèviament apreses i impediria la seva inhibició o el seu reemplaçament per conductes més adaptatives (Saxena i Rauch, 2000; Pauls et al., 2014). Cal mencionar que diferents bucles CSTC regulen diferents seqüències de comportament. Per exemple, el circuit sensoriomotor regula les respostes automàtiques i la transició entre els comportaments dirigits a un objectiu i els comportaments habituals, el circuit cognitiu dorsal regula diverses funcions executives, mentre que el circuit cognitiu ventral contribueix a la preparació motora i la inhibició de resposta i el circuit límbic regula el processament emocional i afectiu.

D'acord amb el model CSTC del TOC, estudis neuroquímics i de neuroimatge mostren que en el TOC es produeix un increment de la neurotransmissió glutamatèrgica i dopaminèrgica en les vies fronto-estriatals i una disminució en la neurotransmissió serotoninèrgica i GABAèrgica en les vies fronto-límbiques (Graat, Figee i Denys, 2017). La implicació d'aquests neurotransmissors en el TOC també s'ha vist recolzada per estudis genètics (Taylor, 2012; 2015; Stewart et al., 2013; Mattheisen, et al., 2015; Noh et al., 2017). Entre ells, el rol del glutamat sembla ser especialment rellevant en el TOC (Bozorgmehr, Ghadirivasfi i Shahsavand-Ananloo, 2017; Fernandez, Leckman i Pittenger, 2018). De fet, models computacionals han suggerit que el TOC podria sorgir de la hiperactivació en la neurotransmissió glutamatèrgica que incrementaria la capacitat de certs nodes cerebrals d'atraure la connectivitat funcional (i.e. *attractors*) i això causaria que al cervell li costés més sortir d'estats cognitius molt estables i dificultaria la transició a nous estats (Rolls, Loh i Deco, 2008). Si aquestes alteracions tinguessin lloc en diferents

regions cerebrals, podrien conduir a l'expressió de diferents símptomes (Rolls, Loh i Deco, 2008), fet que concorda amb l'heterogeneïtat clínica del TOC.

### 1.3. Heterogeneïtat simptomatològica en el TOC: el model multidimensional

Tot i que el TOC es considera una única entitat nosològica, aquest trastorn es caracteritza per la seva heterogeneïtat clínica, fins al punt que dos pacients amb el mateix diagnòstic poden presentar símptomes completament diferents (Mataix-Cols, Rosario-Campos i Leckman, 2005). En la recerca, estudiar el TOC com una única entitat ha comportat resultats heterogenis i ha contribuït a la idea que diferents símptomes del TOC podrien relacionar-se amb alteracions neurobiològiques distintes. Per tant, tenir en compte aquesta heterogeneïtat clínica en la recerca podria facilitar la definició de subgrups més homogenis de pacients i contribuir en la identificació d'endofenotips més robusts.

En aquest sentit, el model multidimensional té en compte aquesta heterogeneïtat al classificar els símptomes en diferents dimensions clíniques. Aquestes dimensions es poden entendre com un espectre de síndromes potencialment sobreposades que poden coexistir en qualsevol pacient (Mataix-Cols, Rosario-Campos i Leckman, 2005). En els darrers 30 anys, diversos estudis han procurat definir dimensions que expliquin un llarg percentatge de la variància clínica mitjançant anàlisis factorials tant en mostres adultes com pediàtriques de TOC (Baer, 1994; Mataix-Cols, Rosario-Campos i Leckman, 2005; Bloch et al., 2008; Stewart et al., 2008; Nikolajsen, Nissen i Thomsen, 2011; Brakoulias et al., 2013). Al llarg d'aquests, s'han definit les següents dimensions de forma consistent: *ordre/simetria, contaminació/neteja, acumulació, agressió/comprovació* (també coneguda com a *dubte/comprovació*) i *pensaments inacceptables/taboo* (també anomenats *obsessions sexuals/religioses* o símptomes *obsessius*) (Brakoulias et al., 2013). Tot i això, en aquests estudis el nombre de dimensions i els símptomes continguts en cada una d'elles ha estat variable. En conseqüència, els qüestionaris de símptomes que s'han desenvolupat a partir d'aquests també són diferents entre si, fet que inevitablement dificulta la comparació entre estudis implementant diferents instruments. Malgrat això, l'aproximació multidimensional ha permès descriure

variacions entre dimensions a nivell sociodemogràfic, neuropsicològic, clínic, genètic i de neuroimatge.

### 1.4. Resum de la investigació prèvia en els correlats estructurals i de connectivitat funcional del TOC

Estudis de RM estructural avaluant el TOC com una entitat clínica homogènia:

Les troballes més consistents al llarg de diferents meta-anàlisis de morfometria basada en vòxel han estat alteracions estructurals en regions fronto-estriades. S'ha reportat un increment de volum de substància grisa (SG) en el nucli lenticular, principalment al putamen i sovint estenent-se al caudat i al tàlem. Aquest increment s'ha associat positivament amb l'edat i sembla ser més prominent en adults i en pacients medicats (Radua i Mataix-Cols, 2009; Radua et al., 2010; Peng et al., 2012; de Wit et al., 2014; Eng, Sim i Chen, 2015; Hu et al., 2017; Picó-Pérez et al., 2020). Contràriament, s'han reportat reduccions de volum de SG en regions prefrontals (Radua i Mataix-Cols, 2009; Radua et al., 2010; Peng et al., 2012; de Wit et al., 2014; Hu et al., 2017; Picó-Pérez et al., 2020), tot i que l'evidència suggereix que aquesta característica podria ser comuna amb altres trastorns psiquiàtrics (Radua et al., 2010; Goodkind et al., 2015) i també vulnerable a l'efecte de l'edat i la medicació (de Wit et al., 2014). Mega- i meta-anàlisis estudiant el volum subcortical també han reportat que l'increment del nucli pàl·lid és específic d'adults amb TOC, junt amb la disminució de l'hipocamp (Fouche et al., 2017; Boedhoe et al., 2017), mentre que l'increment en el tàlem sembla específic d'infants amb TOC (Boedhoe et al., 2017). Per altra banda, mega- i meta-anàlisis de gruix i superfície cortical han mostrat una disminució del gruix cortical d'àrees parietals tant en adults com en infants amb TOC (Fouche et al., 2017; Boedhoe et al., 2018), sovint estenent-se a regions temporals en adults (Fouche et al., 2017; Boedhoe et al., 2018) i occipitals en infants (Boedhoe et al., 2018). Finalment, mega- i meta-anàlisis centrades en la substància blanca (SB) han mostrat alteracions força extenses implicant circuits fronto-estriats, fronto-parietals, l'estrat sagital, la radiació talàmica posterior i el cos callós (Piras et al., 2013; Peng et al., 2012; de Wit et al., 2014; Radua et al., 2014; Piras et al., 2019).

Estudis de RM estructural implementant el model multidimensional:

Els estudis estructurals que han utilitzat un model multidimensional han donat resultats força heterogenis. Tot i això, unint les troballes que s'han obtingut investigant la SG i SB des de diferents metodologies (i.e. morfometria basada en vòxel, volum subcortical, gruix i superfície cortical i imatge per tensors de difusió), podem extreure'n algunes conclusions. Les dimensions de *dubte/comprovació* i *contaminació/neteja* s'han associat consistentment a alteracions en la SG i SB dels ganglis basals (van den Heuvel et al., 2009; Alvarenga et al., 2012; Lázaro et al., 2014b; Kim et al., 2015). A més, els símptomes de *dubte/comprovació* també s'han relacionat amb canvis en la SG i SB de regions temporals (van den Heuvel et al., 2009; Okada et al., 2015; Fouche et al., 2017; Yagi et al., 2017), fet que concorda amb el vincle establert entre aquests símptomes i la dificultat de recordar si s'ha completat una acció (Rachman, 2002; van den Heuvel, 2009; Leopold i Backenstrass, 2015). Per altra banda, els símptomes *obsessius* o *sexuals/religiosos* s'han vinculat amb alteracions en la SG i SB dels girs cingulat i supramarginal (Alvarenga et al., 2012; Koch et al., 2012; Fouche et al., 2017; Yagi et al., 2017), que contribueixen al control cognitiu. Finalment, la dimensió d'*ordre/simetria* s'ha relacionat amb canvis en la SG i SB de regions fronto-occipitals (Valente et al., 2005; Okada et al., 2015; Boedhoe et al., 2017; Fouche et al., 2017), fet que podria explicar els dèficits en el processament visual observat en individus amb aquests símptomes.

Estudis de connectivitat funcional en repòs avaluant el TOC com una entitat clínica homogènia:

La majoria d'estudis que han examinat alteracions de la connectivitat funcional en TOC han utilitzat aproximacions basades en *seeds* que impliquen la selecció d'una regió cerebral a priori. Tot i que aquests estudis han contribuït a la formulació del model CSTC del TOC, aquesta aproximació limita les àrees estudiades i la comparació entre estudis, fet que podria explicar els resultats heterogenis als que ha donat peu. Per exemple, en individus amb TOC la connectivitat entre l'estriat ventral i el còrtex obritofrontal (COF) s'ha vist principalment augmentada (Harrison et al., 2009; Sakai et al., 2011; Jung et al., 2013; Harrison et al., 2013) però també disminuïda (Posner et al., 2014). De forma

similar, la connectivitat entre l'estriat dorsal i regions frontals s'ha descrit tant com a augmentada com a disminuïda, tant en adults com en infants amb TOC (Harrison et al., 2009; Fitzgerald et al., 2011; Posner et al., 2014; Bernstein et al., 2016). L'efecte de la medicació o d'altres factors confusors pot haver jugat un rol clau en l'heterogeneïtat d'aquests resultats. Per altra banda, una meta-anàlisi d'estudis basats en *seeds* va reportar hipoconnectivitat en la xarxa fronto-parietal, la xarxa de saliència i la xarxa neuronal per defecte (DMN, per les sigles en anglès), i també entre elles. A més, també va mostrar connectivitat aberrant (i.e. sense direcció específica) en aquestes xarxes, implicant regions subcorticals com l'estriat i el tàlem medial-dorsal (Gürsel et al., 2018). Altres estudis aplicant anàlisis de components independents o metodologies basades en teoria de grafs han aportat evidència de la implicació de xarxes funcionals a gran escala en el TOC (Zhang et al., 2011; Beucke et al., 2013; Anticevic et al., 2014; Göttlich et al., 2014; Hou et al., 2014; Shin et al., 2014; Tian et al., 2015; Jung et al., 2017; Gürsel et al., 2020). Tot i això, la direccionalitat dels resultats ha estat molt variable, possiblement degut a l'ús de mostres petites i metodologies diferents.

<u>Estudis de connectivitat funcional en repòs implementant el model multidimensional</u>:

Només dos estudis han examinat les diferències de connectivitat funcional entre dimensions. El primer d'ells es va centrar en la connectivitat de la DMN i va observar que la connectivitat entre aquesta i el gir frontal mig s'associava positivament amb la dimensió de *contaminació/neteja* però negativament amb *obsessió/comprovació.* A més, aquesta darrera dimensió va correlacionar positivament amb la connectivitat entre la DMN i diverses regions frontals, temporals i occipitals, mentre que la dimensió d'*ordre* es va associar positivament amb la connectivitat entre la DMN i els girs cuneus, lingual i calcarí (Jang et al., 2010). El segon estudi va examinar la connectivitat de l'estriat ventral i dorsal i va trobar que els símptomes d'*agressió* correlacionaven positivament amb la connectivitat funcional entre l'estriat ventral i el còrtex prefrontal (CPF) ventromedial però negativament amb la connectivitat entre l'estriat ventral i l'amígdala. Per altra banda, els símptomes *sexuals/religiosos* s'associaren positivament amb la connectivitat entre l'estriat ventral i el còrtex insular, mentre que els símptomes d'*acumulació* es relacionaren positivament amb la connectivitat entre l'estriat ventral i el cíngol

subgeniculat/COF medial (Harrison et al., 2013). Tot i això, aquests estudis no només van diferir en la selecció de *seeds* sinó també en la forma de quantificar les dimensions, fet que dificulta encara més les comparacions.

### 1.5. Una perspectiva de desenvolupament del TOC: des de l'expressió subclínica fins a l'expressió clínica dels símptomes

La presència d'obsessions i compulsions no és exclusiva del TOC. En nens sans d'entre 2 i 5 anys s'han descrit comportaments molt similars i que també poden agrupar-se en dimensions (Evans et al., 1997; Evans, Lewis i Iobst, 2004; Pietrefesa i Evans, 2007). Actualment, no s'ha pogut vincular la presència d'aquests comportaments en la infància amb el posterior desenvolupament del TOC, però en ambdós casos els símptomes s'han descrit com un mecanisme per a reduir l'ansietat i s'han relacionat amb problemes en la inhibició de resposta i la flexibilitat cognitiva (Evans, Lewis i Iobst, 2004; Pietrefesa i Evans, 2007). Es considera que a partir dels 7 anys aquests comportaments disminuirien degut al neurodesenvolupament, però estudis epidemiològics mostren que la prevalença de símptomes obsessius-compulsius subclínics és del 8% en nens d'11 anys i d'entre el 13% i el 28% en adults (Fullana et al., 2009; Ruscio et al., 2010). Tot i que els circuits CSTC continuen madurant fins a l'inici de l'edat adulta, sembla que els símptomes no es limiten a períodes crítics del neurodesenvolupament. De fet, en adults amb símptomes subclínics s'han detectat alteracions estructurals en el cervell molt similars a les trobades de forma consistent en pacients amb TOC (Kubota et al., 2016; 2019). Tot i que desconeixem les causes que durien a l'aparició i manteniment de símptomes subclínics, és possible que en alguns casos la maduració dels circuits CSTC contribueixi a resoldre'ls, mentre que en altres aquests processos de maduració es vegin afectats per predisposició genètica o per factors ambientals, resultant en símptomes obsessius-compulsius subclínics.

De la mateixa manera, tampoc es coneix la relació entre símptomes subclínics i el TOC, però s'ha demostrat que la presència d'obsessions i compulsions als 11 anys predisposa a desenvolupar TOC en l'edat adulta (Fullana et al., 2009) i que aquesta probabilitat augmenta amb el nombre de símptomes (Ruscio et al., 2010). Per tant, és possible que

aquests símptomes siguin un precursor del TOC en alguns casos, com podria ser en els descendents d'individus amb TOC. Això concorda amb estudis retrospectius en els quals s'ha reportat que pacients amb TOC presentaven símptomes subclínics entre 5 i 7 anys abans del diagnòstic (Coles, Johnson i Schubert, 2011; Thompson et al., 2020) i també amb estudis familiars on s'ha vist que aquests símptomes són més prevalents en familiars de primer grau de pacients amb TOC (Black i Gaffney, 2008). A més, tant en mostres clíniques com subclíniques, les dimensions simptomatològiques observades en adults s'han relacionat amb els mateixos precursors simptomàtics durant la infància (Fullana et al., 2009), el que suggereix que el TOC i els símptomes subclínics podrien compartir una mateixa base neurobiològica que es podria estudiar amb l'aproximació multidimensional. Per tant, tenint en compte les similituds en el tipus de símptomes, i possiblement també en la neurobiologia i neuropsicologia, els símptomes obsessius-compulsius subclínics es podrien considerar part de l'espectre TOC (Nestadt et al., 2000; Black i Gaffney, 2008) i el seu estudi podria contribuir a una millor comprensió sobre el desenvolupament del TOC, així com en la caracterització d'individus en risc de desenvolupar aquest trastorn.

### 1.6. Motivació de l'estudi

Els símptomes obsessius-compulsius subclínics són relativament comuns en la població general, tant en joves com adults (Fullana et al., 2009; Ruscio et al., 2010) i, tot i que difereixen del TOC en el grau de severitat i discapacitat, comparteixen una fenomenologia similar. Per tant, els símptomes subclínics es podrien considerar part de l'espectre del TOC. En aquesta línia, l'evidència indica que aquests símptomes podrien ser precursors del TOC en alguns casos i que, a més, les dimensions de símptomes observades en adults, tant a nivell clínic com subclínic, es vinculen al mateix perfil de símptomes precursors en la infància (Fullana et al., 2009; Ruscio et al., 2010), fet que recolza la idea que els símptomes obsessius-compulsius clínics i subclínics podrien compartir les mateixes marques neurobiològiques que es podrien explorar des d'una perspectiva multidimensional.

Els diversos estudis de neuroimatge utilitzant el model multidimensional han donat resultats força heterogenis que possiblement es poden atribuir a múltiples factors confusors. En infants amb TOC, aquestes aproximacions han estat molt escasses i s'han fet en mostres molt petites de pacients. En contrast, l'estudi dels correlats estructurals i funcionals de diferents dimensions de símptomes subclínics en una mostra robusta de nens sans permetria caracteritzar els correlats neurals d'aquests símptomes sense la interferència de l'efecte de la medicació, la cronicitat del trastorn o les comorbiditats i tenint en compte diferents perfils de símptomes. En aquestes aproximacions és clau, per una banda, analitzar els resultats a nivell de tot el cervell, ja que la recerca del TOC ha demostrat la implicació d'àrees molt diverses i distants en el trastorn i, per altra banda, tenir en compte els canvis en el neurodesenvolupament que tenen lloc durant la infància i que difereixen entre sexes.

A més, considerant els factors genètics i les alteracions en la neurotransmissió que s'han descrit en el TOC, els correlats de neuroimatge dels símptomes obsessius-compulsius subclínics es podrien complementar amb anàlisis d'imatge transcriptòmica que permetrien identificar la base genètica de les alteracions. Això podria aportar una visió més integrada del desenvolupament d'aquests símptomes. Si addicionalment es disposa de dades genòmiques dels participants, aquestes es podrien combinar amb els resultats de les anàlisis d'imatge transcriptòmica per tal de detectar polimorfismes que predisposarien a alteracions neurobiològiques associades als símptomes subclínics.

## 2. OBJECTIUS

L'objectiu general d'aquesta tesi és aportar informació sobre els correlats neurobiològics dels símptomes obsessius-compulsius subclínics en una mostra de nens sans, amb l'esperança que aquesta informació pugui ser eventualment vinculada al desenvolupament del TOC i, per tant, servir per caracteritzar individus en risc. Amb aquest objectiu en ment, hem utilitzat diferents modalitats i tècniques de neuroimatge per tal d'avaluar les potencials associacions entre símptomes i trets cerebrals. A més, en

el darrer estudi hem combinat dades de neuroimatge i genètica per tal d'aportar una visió més integrada de l'inici dels símptomes.

**Estudi 1:**

- Identificar característiques volumètriques del cervell associades als símptomes subclínics totals i dimensionals (i.e. *dubte-comprovació, acumulació, obsessions* i *ordre*) en una gran mostra de nens sans.
- Avaluar els potencials efectes moduladors de l'edat i el sexe en la relació entre anatomia cerebral i els símptomes.

**Estudi 2:**

- Identificar canvis en la connectivitat funcional global associats als símptomes subclínics totals i dimensionals (i.e. *dubte-comprovació, acumulació, obsessions* i *ordre*) en una gran mostra de nens sans.
- Avaluar els patrons de connectivitat funcional que sorgeixen de les regions que presenten connectivitat global alterada, així com les associacions que aquests patrons estableixen amb els símptomes subclínics totals i dimensionals.
- Avaluar els potencials efectes moduladors de l'edat i el sexe en la relació entre la connectivitat funcional cerebral i símptomes.

**Estudi 3:**

- Identificar *attractors* cerebrals (i.e. nodes amb propietats atractives alterades que fan que la connectivitat funcional dinàmica convergeixi repetidament en ells) associats als símptomes subclínics totals i dimensionals (i.e. *dubte-comprovació, acumulació, obsessions* i *ordre*).
- Analitzar la base genètica dels *attractors* associats als símptomes mitjançant imatge transcriptòmica.

- Detectar polimorfismes d'un sol nucleòtid (SNPs, per les seves sigles en anglès) que modulin l'associació entre la connectivitat dels *attractors* i els símptomes mitjançant les dades genòmiques de la nostra mostra.

## 3. MÈTODES

### 3.1. Descripció de la mostra i avaluació psicomètrica

Els tres estudis d'aquesta tesi comparteixen la mateixa mostra de nens sans d'entre 8 i 12 anys recollida en el marc del projecte BREATHE (Comissió Europea: FP7-ERC-2010-AdG, ID 268479). De la mostra de neuroimatge adquirida inicialment (N=263), es varen excloure alguns subjectes per criteris de qualitat d'imatge o per falta de dades. El 1r estudi va tenir una mostra de 255 nens. Donat que 25 d'aquests presentaven símptomes subclínics de trastorn per dèficit d'atenció i hiperactivitat (TDAH), es van repetir les anàlisis excloent aquests subjectes per tal de confirmar que els resultats obtinguts no variaven. Contràriament, en el 2n i 3r estudi, on es tenia una mostra de 252 subjectes, es van excloure directament aquests 25 nens, resultant en 227 participants.

L'avaluació psicomètrica va constar de tres qüestionaris i una entrevista clínica. Per tal de quantificar els símptomes obsessius-compulsius subclínics, els nens van emplenar la versió pediàtrica de l'inventari d'obsessions i compulsions (OCI-CV, per les sigles en anglès), que permet mesurar 6 dimensions de símptomes (*i.e. dubte-comprovació, acumulació, neutralització, ordre, obsessions i neteja*) i obtenir una puntuació total en sumar totes les puntuacions dimensionals (versió espanyola: Rosa-Alcázar et al., 2014). Per altra banda, els pares o tutors dels participants varen emplenar el qüestionari de qualitats i dificultats (SDQ, per les sigles en anglès), que quantifica les dificultats comportamentals i el comportament prosocial (versió espanyola: sdqinfo.com). Complementàriament, els professors dels participants van completar l'escala de puntuació de símptomes TDAH-IV (ADHD-RS-IV, per les sigles en anglès), que mesura la falta d'atenció i la hiperactivitat/impulsivitat (versió espanyola: Servera i Cardo, 2007). Finalment, tots els participants van passar una entrevista clínica amb un psicòleg o

psiquiatre de l'equip, fet que va permetre confirmar que cap participant complia criteris diagnòstics per un trastorn psiquiàtric. Malgrat això, tal com hem dit prèviament, 25 nens presentaven símptomes TDAH subclínics.

### 3.2. Tècniques de neuroimatge

RM estructural: La RM utilitza les propietats magnètiques dels protons d'hidrogen, presents en les molècules d'aigua del cos humà, per generar imatges detallades de qualsevol teixit, en aquest cas, del cervell. En el 1r estudi, les imatges estructurals es van utilitzar en l'anàlisi de morfometria basada en vòxel, implementada al programa SPM (SPM8; The Wellcome Department of Imaging Neuroscience, London, UK), que permet quantificar el volum regional de SG i SB. Aquesta informació ens va permetre detectar canvis volumètrics associats als símptomes obsessius-compulsius subclínics totals i dimensionals. En el 2n i 3r estudi les imatges estructurals van servir per corregistrar les imatges funcionals, ja que gràcies a la seva millor resolució permeten estudiar la funció cerebral amb major especificitat anatòmica.

RM funcional: Aquesta seqüència mesura indirectament l'activitat del cervell a partir del senyal dependent del nivell d'oxigenació de la sang (BOLD, per les sigles en anglès). A partir d'aquest senyal, en el 2n i 3r estudi d'aquesta tesi vam poder estudiar la connectivitat funcional del cervell en repòs, que captura patrons sincrònics de fluctuacions espontànies del senyal BOLD i permet copsar l'organització funcional dels sistemes cerebrals a gran escala (Raichle, 2010).

En el 2n estudi vam utilitzar les imatges funcionals per estudiar la connectivitat funcional global mitjançant l'anàlisi de contrast de connectivitat intrínseca, implementada en la CONN *toolbox* (Whitfield-Gabrieli i Nieto-Castanon, 2012; CONN Toolbox v17; www.nitrc.org/projects/conn). A continuació, vàrem estudiar la relació entre els símptomes totals i dimensionals i la connectivitat funcional global i, amb els resultats significatius, vam fer anàlisis *post-hoc* basades en *seeds* per tal de caracteritzar els patrons específics de connectivitat d'aquestes regions que s'associaven amb els símptomes totals i dimensionals. De nou, aquestes anàlisis es van fer mitjançant la CONN

*toolbox* (Whitfield-Gabrieli i Nieto-Castanon, 2012; CONN Toolbox v17; www.nitrc.org/projects/conn).

Contràriament, en el 3r estudi, vam utilitzar les imatges funcionals per detectar *attractors* cerebrals. Aquesta anàlisi es va fer mitjançant l'aproximació *Stepwise Functional Connectivity* basada en teoria de grafs i descrita a Diez i Sepulcre (2018), que es pot implementar mitjançant scripts de MATLAB (MATLAB v2020b; The Math Works Inc, Natick, MA). És important destacar que aquest mètode utilitza l'aproximació *sliding window* per mesurar la connectivitat funcional dinàmica i, a més, permet segregar la connectivitat en local i distant segons la seva modularitat. Per tant, quan utilitzem els termes local i distant en aquesta tesi, ens referim a distàncies basades en la topologia de la xarxa cerebral i no a distància euclidiana dins del cervell (Diez i Sepulcre, 2018). Finalment, mitjançant l'SPM (SPM12; The Wellcome Department of Imaging Neuroscience, London, UK), vam estudiar la relació entre la connectivitat dinàmica *attractora* i els símptomes tant totals com dimensionals.

### 3.3. Avaluació de factors del neurodesenvolupament

L'efecte del sexe, l'edat i la interacció sexe-edat es va estudiar dividint la mostra en subgrups (sexe: nenes vs. nens; edat: menors vs. majors de la mediana de l'edat de la mostra; sexe-edat: nenes menors vs. resta, nenes majors vs. resta, nens menors vs. resta i nens majors vs. resta) i comparant les correlacions entre símptomes i resultats d'imatge en cada un d'ells. Totes aquestes correlacions es van contrastar mitjançant transformacions de Fisher. Addicionalment, la interacció entre edat i símptomes també es va mesurar amb models de regressió lineal. Aquestes anàlisis es van fer mitjançant el programa SPSS (SPSS 23; IBM Corp, Armonk, NY).

### 3.4. Interacció neuroimatge-expressió genètica

En el 3r estudi vam utilitzar una aproximació metodològica que ens va permetre explorar la base genètica dels *attractors* associats a símptomes obsessius-compulsius subclínics i també detectar SNPs que, en la nostra mostra, modulaven la relació entre connectivitat

*attractora* i símptomes. En primer lloc, vam comparar la similitud espacial entre els nostres mapes de connectivitat *attractora* associada als símptomes amb l'atles Allen Human Brain Atlas (AHBA) d'expressió gènica (Hawrylycz, et al., 2012; Allen Human Brain Atlas: human.brain-map.org). Això ens va permetre obtenir una llista de gens que s'expressen en les regions corticals o subcorticals on prèviament havíem detectat *attractors* associats als símptomes. En segon lloc, amb aquests gens vam fer una anàlisi d'enriquiment al portal Gene Ontology (Ashburner et al., 2000; The Gene Ontology Consortium, 2019; Gene Ontology: geneontology.org), fet que ens va permetre detectar si s'associaven a processos biològics específics de forma significativa. En tercer lloc, mitjançant el portal GTEx (The GTEx Consortium, 2013; GTEx Portal V8: gtexportal.org), vam localitzar els SNPs que modificaven l'expressió d'aquests gens en les regions on havíem detectat *attractors* associats als símptomes. En quart lloc, vam filtrar aquestes SNPs per desequilibri de lligament mitjançant l'eina SNPclip (Machiela i Chanock, 2015; SNPClip: ldlink.nci.nih.gov/?tab=snpclip), fet que va reduir significativament el nombre de SNPs, i vam extreure les seves dosis de les dades genòmiques de la nostra mostra mitjançant el programa PLINK V1.9 (Purcell et al., 2007; PLINK V1.9: cog-genomics.org/plink/1.9). Finalment, dividint la nostra mostra en funció de la presència de l'al·lel minoritari de cada SNP (tenir vs. no tenir còpies de l'al·lel minoritari), vam estudiar si l'associació entre connectivitat *attractora* i els símptomes variava en funció d'aquestes variants genètiques amb el SPSS (SPSS 23; IBM Corp, Armonk, NY).

## 4. RESULTATS

### Estudi 1:

- Els símptomes d'*ordre* s'associen negativament al volum de SG del caudat ventral. Aquesta troballa és específica dels nens (i.e. sexe masculí) menors de 10 anys.
- Els símptomes *obsessius* s'associen negativament al volum de SG i SB del pol temporal dret.

- Els símptomes d'*acumulació* correlacionen positivament amb els volums de SG i SB del gir frontal inferior esquerre. L'associació entre aquests símptomes i el volum de SG del gir frontal inferior esquerre és específica dels nens i nenes majors de 10 anys.
- Els símptomes de *dubte-comprovació* correlacionen positivament amb els volums de SB del fascicle fronto-occipital inferior dret (FFOI) i del istme del cos callós i, a nivell de tendència, negativament amb els volums de SG del gir frontal mig, incloent el CPF ventrolateral.

**Estudi 2:**

- Els símptomes obsessius-compulsius totals s'associen negativament amb la connectivitat funcional global del putamen ventral i del tàlem medial-dorsal esquerres.
- Els símptomes d'*ordre* s'associen negativament amb la connectivitat funcional entre el putamen ventral esquerre i regions sensoriomotores (i.e. estriat dorsal esquerre, gir precentral dret, gir supramarginal esquerre i opercle Rolandic/gir temporal superior esquerre).
- Els símptomes *obsessius* s'associen negativament amb la connectivitat funcional entre el putamen ventral esquerre i regions límbiques (i.e. hipocamp dret i tàlem i amígdala esquerres).
- Els símptomes d'*acumulació* s'associen negativament amb la connectivitat funcional entre el putamen ventral i el tàlem medial-dorsal i entre el tàlem medial-dorsal i un ampli patró de regions corticals i subcorticals (i.e. estriat bilateral, nucli subtalàmic dret, gir frontal mig esquerre i els solcs cuneus i calcarí bilaterals). La correlació entre aquests símptomes i la connectivitat entre el tàlem medial-dorsal i el nucli subtalàmic és significativament més negativa en nenes, mentre que l'associació amb la connectivitat entre el tàlem medial-dorsal i regions corticals posteriors (i.e. solcs cuneus i calcarí) és significativament més negativa en nenes majors de 10 anys.
- Els símptomes de *dubte-comprovació* s'associen negativament amb la connectivitat funcional entre el tàlem medial-dorsal i l'ínsula anterior esquerra.

**Estudi 3:**

- Els símptomes obsessius-compulsius totals s'associen negativament a un *attractor* local situat al putamen ventral esquerre, i positivament a dos *attractors* distants localitzats a l'àrea motora suplementària (AMS) bilateral i a l'hipocamp esquerre. Els gens expressats en l'*attractor* de l'hipocamp contribueixen significativament a diversos processos biològics, entre ells, la neurotransmissió glutamatèrgica, la resposta a la por i l'aprenentatge.
- Els símptomes d'*ordre* correlacionen positivament amb un *attractor* distant situat al còrtex parietal superior dret. Els gens expressats en aquesta regió participen significativament a diversos processos biològics, entre ells, la transmissió de l'impuls nerviós i el transport cel·lular.
- L'associació entre símptomes totals i la connectivitat *attractora* distant de l'hipocamp esquerre només és significativa en nens que tenen còpies de l'al·lel minoritari C en els SNPs rs7613638 del gen GRM7, rs11145747 del gen GNAQ i rs1994904 del gen PARVA.
- La correlació entre símptomes d'*ordre* i la connectivitat *attractora* distant del còrtex parietal superior dret només és significativa en nens que tenen còpies de l'al·lel minoritari T en el SNP rs2143290 del gen ATP1B1 i en els que no tenen còpies de l'al·lel minoritari C en el SNP rs6490097 del gen TESC.

## 5. DISCUSSIÓ

### 5.1. Interpretació dels resultats

En aquesta secció proposem interpretacions pels resultats que hem trobat, primer centrant-nos en els dimensionals i, després, en els totals.

Símptomes d'ordre:

L'associació negativa entre els símptomes d'*ordre* i el volum de SG del caudat ventral no és sorprenent, ja que aquesta regió forma part del circuit CSTC cognitiu ventral i les

funcions que aquest regula s'han trobat alterades en TOC (Britton et al., 2010). El fet que aquesta disminució fos significativa en nens menors de 10 anys podria indicar alteracions en el neurodesenvolupament, ja que el caudat augmenta de volum durant la infància (Fareri, Martin i Delgado, 2008), o bé reflectir que el desenvolupament més lent d'aquesta regió en nens, comparat amb nenes de la mateixa edat (Giedd et al., 1996), pot predisposar a símptomes d'*ordre*. Aquesta interpretació és congruent amb la major incidència de TOC pediàtric en nens (Ruscio et al., 2010), amb l'inici temprà dels símptomes d'*ordre* i amb l'alta prevalença d'aquests símptomes en TOC pediàtric (Uguz et al., 2006; Labad et al., 2008; Nakatani et al., 2011; Kichuk et al., 2013).

Tot i això, els nostres resultats funcionals apunten al fet que aquesta dimensió s'associa a menor connectivitat en el circuit CSTC sensoriomotor i a un increment de les propietats *attractores* al còrtex parietal superior dret. Fet que, per una banda, podria alterar la capacitat de transicionar entre seqüències motores, afavorint la repetició de moviments o accions i, per altra banda, podria modificar el processament visuoespacial i atencional flexible, així com les funcions executives, predisposant a l'aparició de símptomes d'*ordre.* Aquestes troballes també són congruents amb estudis previs de neuroimatge (van den Heuvel et al., 2009; Subirà et al., 2015) i neuropsicologia (McGuire et al., 2014; Bragdon, Gibb i Coles, 2018).

A més, en la nostra mostra vàrem detectar que la presència de l'al·lel minoritari T en el SNP rs2143290 del gen ATP1B1 actuava com a factor de risc en l'aparició de l'*attractor* del còrtex parietal superior dret associat als símptomes d'*ordre*, mentre que la presència de l'al·lel minoritari C en el SNP rs6490097 del gen TESC actuava com a factor protector. Aquests gens contribueixen al manteniment de gradients electroquímics en la cèl·lula mitjançant la regulació de l'ATPasa Na+/K+ i l'intercanviador 1 de Na+/H+. Aquests gradients són claus per la correcta neurotransmissió glutamatèrgica, per tant, polimorfismes en aquests gens podrien augmentar o reduir el risc a la hiperactivació de la neurotransmissió glutamatèrgica, el que augmentaria les propietats *attractores* del còrtex parietal superior i predisposaria als símptomes d'*ordre*.

Símptomes obsessius:

Els símptomes *obsessius* s'associen principalment a canvis estructurals i funcionals en regions pròpiament límbiques (i.e. hipocamp, tàlem, amígdala) o del sistema límbic estès (i.e. pol temporal). Tenint en compte les funcions mediades per aquestes regions, alteracions en aquestes àrees podrien causar problemes en el processament de les emocions i l'ansietat, així com en la cognició moral, que predisposarien a l'aparició de símptomes *obsessius*. A nivell neuropsicològic, aquesta interpretació té sentit en tant que s'ha trobat que aquesta dimensió s'associa amb un augment de la por i de l'estimació de les amenaces (Via et al., 2014) i també amb un increment en la importància i control dels pensaments i sentiments de culpa, que sovint tenen un component moral (Wheaton et al., 2010; Brakoulias et al., 2014). De la mateixa manera, els nostres resultats també són congruents amb les troballes d'estudis previs de neuroimatge en mostres clíniques i subclíniques (Alonso et al., 2013; Harrison et al., 2013; Subirà et al., 2013; Via et al., 2014).

Símptomes d'acumulació*:*

La relació entre aquests símptomes i l'increment de volum de SG i SB al gir frontal inferior, així com la reducció en la connectivitat entre el putamen ventral i el tàlem medial dorsal, apunten a la implicació del circuit CSTC cognitiu ventral en la manifestació de símptomes d'*acumulació*. Això és congruent amb troballes neuropsicològiques i de neuroimatge observades en pacients de TOC amb aquests símptomes (Roth et al., 2007; Tops i Boksem, 2011; Morein-Zamir et al., 2014) i en pacients amb trastorn d'acumulació (Levy et al., 2019; Suñol et al., 2019). Tot i això, el patró de connectivitat talàmica associada a aquests símptomes sembla indicar la implicació d'altres circuits (i.e. la reducció en la connectivitat amb el CPF dorsolateral apunta a la implicació del circuit CSTC cognitiu dorsal, mentre que la reducció en la connectivitat amb regions occipitals medials sembla implicar a regions fora del circuit CSTC). El rol del CPF dorsolateral s'ha descrit prèviament en pacients amb trastorn per acumulació i l'evidència suggereix que alteracions en aquesta regió podrien explicar dèficits en funcions executives (Levy et al., 2019; Suñol et al., 2019).

Des de la perspectiva del neurodesenvolupament, trobem que l'augment de volum de SG del gir frontal inferior associat a la dimensió d'*acumulació* és específic dels participants de més de 10 anys. Això podria implicar una disrupció en el neurodesenvolupament, ja que a partir dels 10 anys es produeix una disminució de SG frontal. A més, vàrem trobar que l'associació entre aquests símptomes i certs patrons de connectivitat talàmica eren específics de les nenes o de nenes majors de 10 anys. Per tant, podem interpretar que les nenes preadolescents poden ser més vulnerables als símptomes d'*acumulació.* De fet, estudis previs han reportat que aquesta dimensió té un major component familiar que altres dimensions i que correlaciona en major mesura en díades de mares a filles i entre germanes (Hasler et al., 2007; Samuels et al., 2008; Taberner et al., 2009). A més, un estudi de bessons va demostrar que els factors ambientals contribuïen en el desenvolupament de símptomes d'*acumulació* en noies de 15 anys però no en cap altre grup d'edat o sexe (Ivanov et al., 2017). Així doncs, les nostres troballes poden ser el primer correlat neurobiològic del vincle que s'ha reportat a nivell clínic i epidemiològic entre la dimensió d'*acumulació* i el sexe femení durant l'adolescència.

Símptomes de dubte-comprovació:

En el cas dels símptomes de *dubte-comprovació* trobem un increment de SB en el FFOI i l'istme del cos callós, fet que podria reflectir una acceleració en la maduració de la SB d'aquestes regions i comportar una alteració en el funcionament de regions frontals (Luders, Thompson i Toga, 2010; Eccher, 2014; Wu et al., 2016). Trobem, també, una disminució, a nivell de tendència, en la SG del CPF ventrolateral que, com hem vist prèviament, podria implicar el mal funcionament del circuit CSTC cognitiu ventral. Aquests resultats concorden amb estudis previs de neuroimatge i neuropsicologia en TOC que han reportat alteracions en la integritat de la SB del FFOI i el cos callós (Garibotto et al., 2010; Zarei et al., 2011; Gruner et al., 2012; Fitzgerald et al., 2014; Koch et al., 2014; Gan et al., 2017) i alteracions en el CPF ventrolateral que s'han associat a disrupcions en la flexibilitat cognitiva (Piras et al., 2015; Vaghi et al., 2017), una funció que s'ha descrit com a alterada en individus amb compulsions de *comprovació* tant a nivell clínic com subclínic (Leopold i Backenstrass, 2015; Snorrason et al., 2016; Cameron

et al., 2020). L'associació entre aquests símptomes i la reducció en la connectivitat entre el tàlem i l'ínsula anterior també podria implicar el circuit CSTC cognitiu ventral, fet que donaria suport a la nostra interpretació. Tot i això, el tàlem i l'ínsula anterior són nodes claus de la xarxa de saliència. Per tant, alteracions en la connectivitat entre aquestes dues àrees podria afectar el correcte processament d'estímuls salients i causar una sensibilitat emocional augmentada a aquests estímuls. Hipotèticament, els símptomes de *comprovació* podrien aparèixer com un mecanisme per afrontar l'ansietat causada pel funcionalment alterat de la xarxa de saliència, fet que concorda amb estudis anteriors vinculant la reducció de la connectivitat entre el tàlem i l'ínsula amb la severitat del TOC (Stern et al., 2012), i associant la major intolerància a la incertesa, observada en aquest grup de pacients, amb alteracions a l'ínsula (Tolin et al., 2003; Simmons et al., 2008; Song et al., 2011).

Símptomes totals:

La reducció de connectivitat global al tàlem medial-dorsal associada als símptomes totals indica una alteració dels circuits CSTC, ja que aquesta regió és el node final de diferents circuits CSTC. Per aquest motiu, no podem determinar si aquesta troballa implica un circuit específic o diferents bucles del sistema CSTC. Contràriament, la mateixa troballa al putamen ventral suggereix una disminució en la comunicació interna del circuit CSTC cognitiu ventral, ja que aquesta regió connecta el CPF ventrolateral amb el tàlem. Ambdós resultats concorden amb troballes prèvies en mostres de pacients amb TOC (i.e. alteracions en el volum talàmic de joves amb TOC: Rotge et al., 2009; Boedhoe et al., 2017; disminució de la connectivitat al putamen o al circuit cognitiu ventral en adults i joves amb TOC: Harrison et al., 2009; Bernstein et al., 2016; Giménez et al., 2017). Per altra banda, l'associació negativa entre els símptomes i la connectivitat del putamen ventral es va replicar en el 3r estudi. En aquest cas, els símptomes s'associaren a una menor capacitat *attractora* de connectivitat local en aquesta regió, fet que ens demostra que quan estudiem la connectivitat total, la connectivitat local tendeix a eclipsar els patrons distants. Aquesta darrera troballa es pot interpretar com que el putamen perd la seva capacitat de captar connectivitat de regions veïnes, fet que contribuiria a alterar el circuit CSTC cognitiu ventral.

Contràriament, els símptomes totals es van associar a un *attractor* distant en l'AMS, que forma part del circuit CSTC sensoriomotor. Això suggereix que la connectivitat provinent d'àrees subcorticals motores podria convergir i reverberar en aquesta regió causant alteracions en la transició entre moviments i facilitant la repetició d'accions. Aquesta interpretació concorda amb estudis que han demostrat que els efectes inhibitoris de l'estimulació magnètica transcranial repetitiva a baixa freqüència en l'AMS milloren els símptomes TOC (Berlim, Neufeld i Van den Eynde, 2013; Zhou et al., 2017). L'augment de la capacitat *attractora* de l'AMS associat als símptomes totals no té per què contradir la reducció de connectivitat en el bucle sensoriomotor CSTC que vam trobar associada a la dimensió d'*orde*. En aquest context, l'AMS podria segrestar la connectivitat distant causant dèficits en la connectivitat modular del bucle sensoriomotor CSTC i en la connexió entre aquest bucle i el bucle cognitiu ventral, fet que podria predisposar als símptomes d'*ordre*. Esperem que la futura recerca pugui confirmar o desmentir aquesta hipòtesi.

Finalment, els símptomes totals també es van associar a un *attractor* distant en l'hipocamp. Aquest resultat indica que la connectivitat de regions corticals prefrontals o d'associació podria convergir i reverberar en aquesta regió límbica, fet que podria alterar el processament emocional i la integració d'estímuls negatius. Això concorda amb els biaixos de memòria cap a estímuls relacionats amb l'amenaça i amb la tendència evitativa reportada en pacients amb TOC (Muller i Roberts, 2005; Endrass et al., 2011). Tot i que l'estudi de regions límbiques en el TOC és limitat i ha donat resultats heterogenis, la nostra troballa és compatible amb un estudi que va utilitzar una metodologia similar a la nostra i va detectar que la connectivitat local de l'hipocamp, però no la distant, es trobava reduïda en pacients amb TOC (Göttlich et al., 2014). Això podria explicar per què en els nostres estudis hem detectat una disminució en la connectivitat total (i.e. que sobre-representaria la connectivitat local) entre el putamen ventral i l'hipocamp associada als símptomes *obsessius* però en canvi un augment en la connectivitat distant convergint en l'hipocamp associat als símptomes totals. De nou, caldran futurs estudis per confirmar aquesta hipòtesi.

A més, l'increment en les propietats *attractores* distants de l'hipocamp es va relacionar amb la neurotransmissió glutamatèrgica, fet que dona suport a la teoria de Rolls, Loh i

Deco (2008). Per una banda, els gens expressats en aquesta regió es van agrupar en processos biològics relacionats amb la transmissió glutamatèrgica i, per altra banda, vam detectar que la presència de l'al·lel minoritari C en els SNPs rs7613638 del gen GRM7 i rs11145747 del gen GNAQ suposaven factors de risc per aquest *attractor*. El gen GRM7 codifica el receptor glutamatèrgic metabotròpic 7, que regula l'activitat dels receptors glutamatèrgics N-metil-D-aspartat (NMDA) i la transmissió excitatòria en l'hipocamp (Cosgrove et al., 2011; Fisher et al., 2018), mentre que el gen GNAQ codifica la proteïna Gαq que media la senyalització intracel·lular a través de l'activació de receptors units a proteïna Gαq, com els receptors glutamatèrgics metabotròpics del grup I, i contribueix a regular l'activació dels receptors NMDA en l'hipocamp (MacDonald, Jackson i Beazely, 2007). Per tant, polimorfismes en aquests gens podrien incrementar la transmissió glutamatèrgica en l'hipocamp, fet que alteraria la seva plasticitat i capacitat *attractora*, contribuint a biaixos cap a tendències evitatives i memòries negatives. Això concorda amb els canvis comportamentals observats en models animals amb depleció d'aquests gens en l'hipocamp (Fendt et al., 2008; Graham et al., 2015). La presència de l'al·lel minoritari C en el SNP rs1994904 del gen PARVA també actuava com a factor de risc. Aquest gen codifica per l'α-parvina, que s'uneix a l'actina i contribueix a l'adhesió, motilitat i supervivència cel·lular. Aquest gen no s'ha associat amb la transmissió glutamatèrgica o amb el TOC, però un estudi va suggerir que alteracions en els processos de regulació del citoesquelet d'actina i de molècules d'adhesió cel·lular unides a actina, podrien suposar un factor de risc en el desenvolupament del TOC (Yue et al., 2016). Per tant, el vincle entre el gen PARVA i l'increment en les propietats *attractores* de l'hipocamp, possiblement es pugui explicar a través d'aquests processos.

### 5.2. Limitacions

Diverses limitacions metodològiques obliguen a interpretar els resultats presentats en aquesta tesi amb certa cautela. En aquesta secció fem un resum de les limitacions més rellevants que comparteixen tots els estudis presentats.

En primer lloc, els tres estudis són transversals i correlacionals, fet que ens impedeix establir relacions de causalitat entre símptomes i correlats neurobiològics. Donat que

els símptomes subclínics s'han relacionat prèviament amb un major risc de desenvolupar TOC (Fullana et al., 2009), les nostres troballes es podrien considerar, de forma temptativa, com a marcadors de risc pel TOC. Malgrat això, només futurs estudis longitudinals podran discernir si les nostres troballes són veritablement predictives del trastorn o s'associen exclusivament amb els símptomes subclínics.

En segon lloc, donat que el reclutament de participants va succeir en el marc del projecte BREATHE, als nostres estudis els manca un grup de comparació aparellat per edat i sexe i conformat per participants diagnosticats amb TOC. Comparar aquests dos grups ens hauria permès determinar si les característiques neurobiològiques que hem identificat també es troben presents en una mostra pediàtrica de TOC.

En tercer lloc, reclutar exclusivament nens sans té l'avantatge d'evitar els efectes confusors típics de les mostres clíniques. Tot i això, hi ha certs factors confusors que no es van controlar completament i que, per tant, poden haver afegit variabilitat als resultats. Per exemple, els participants van passar només per una entrevista clínica i no vàrem avaluar l'estat puberal ni l'historial mèdic familiar de condicions psiquiàtriques o neurològiques o l'ús de medicació.

Finalment, tot i que hem combinat diferents tècniques, modalitats i dades neurobiològiques, l'avaluació d'altres seqüències de neuroimatge (p. ex. imatge per tensors de difusió o RM funcional basada en tasca), així com la implementació d'altres tècniques d'anàlisis (p. ex. anàlisis de superfície o de gruix cortical) hauria pogut complementar els nostres resultats i ajudar-nos a avançar en la comprensió de les bases neurobiològiques dels símptomes obsessius-compulsius subclínics.

### 5.3. Implicacions per la futura recerca

En aquesta tesi, hem procurat avaluar els símptomes obsessius-compulsius subclínics en nens sans des d'una perspectiva integradora, implementant diferents modalitats i tècniques de neuroimatge i combinant dades de neuroimatge i genètica. Considerant l'escassa literatura científica sobre aquest tema, els tres estudis d'aquesta tesi suposen un pas endavant en la comprensió de les bases neurobiològiques dels símptomes obsessius-compulsius subclínics.

Per concloure aquesta tesi, discutirem possibles línies de recerca futures que, en la nostra opinió, contribuirien a desxifrar el complex vincle entre símptomes obsessius-compulsius subclínics i TOC. En primer lloc, la majoria d'estudis abordant aquesta qüestió, incloent-hi els d'aquesta tesi, són transversals. Per tant, es necessiten futurs estudis longitudinals amb mostres robustes i avaluacions clíniques de seguiment apropiades per tal de determinar si les característiques neurobiològiques que hem trobat associades als símptomes subclínics són realment predictors del desenvolupament del TOC. A més, incloure avaluacions de neuroimatge de seguiment també ajudaria a inferir causalitat entre els canvis cerebrals i clínics.

En segon lloc, partint de la nostra experiència, recomanem l'ús d'enfocaments multidimensionals per avaluar els símptomes obsessius-compulsius. Fins ara, els estudis de neuroimatge estructural i funcional que han utilitzat aquest enfocament han donat resultats relativament heterogenis. Tot i això, molta d'aquesta recerca dóna suport a la noció que, malgrat que algunes característiques neurobiològiques siguin comunes en diferents dimensions de símptomes, també és cert que aquestes dimensions es caracteritzen per alteracions neurobiològiques pròpies i exclusives. Aquest fet és especialment rellevant de cara a futurs estudis longitudinals que pretenguin obtenir marcadors neurobiològics del desenvolupament del TOC, ja que les seves troballes podrien contribuir a la identificació d'individus en risc i a informar el disseny d'estratègies de prevenció. En aquests casos, si s'avaluen els símptomes com un conjunt homogeni es corre el risc de trobar marcadors que només s'associïn als símptomes més ben representats en les mostres de TOC (i.e. *dubte-comprovació* o *neteja*), mentre que no es seria capaç de detectar les característiques que predisposen als símptomes menys prevalents. En conseqüència, les estratègies de prevenció que es podrien derivar d'aquests estudis no resultarien efectives per un gran nombre d'individus amb TOC. Contràriament, l'enfocament multidimensional pot ajudar a identificar marcadors neurobiològics més específics i, per tant, pot servir per dissenyar estratègies de prevenció més personalitzades que beneficiïn a individus amb perfils simptomàtics diferents.

En tercer lloc, a l'avaluar joves amb símptomes obsessius-compulsius és important tenir en compte els diferents factors del neurodesenvolupament com l'edat, el sexe, l'estat

puberal o la presència de símptomes d'altres trastorns del neurodesenvolupament com el TDAH. De la mateixa manera que el model multidimensional, aquest enfocament podria servir per a una millor caracterització dels individus amb aquests símptomes i, per tant, podria contribuir en el disseny d'estratègies de prevenció més personalitzades.

Finalment, considerem que l'estudi de símptomes obsessius-compulsius, tant a nivell subclínic com clínic, es beneficiaria molt de la introducció de metodologies innovadores que permetin avaluar la connectivitat funcional del cervell d'una forma més extensa i completa. En aquesta línia, cal destacar el potencial de les anàlisis de connectivitat funcional dinàmica que no assumeixen que les xarxes cerebrals funcionals són estàtiques i que permet caracteritzar les fluctuacions de la connectivitat al llarg del temps. De forma similar, els trastorns psiquiàtrics s'han vinculat a alteracions en xarxes funcionals de gran escala, implicant regions cerebrals distants entre si, però funcionalment connectades, el que requereix que aquests trastorns s'estudiïn des d'una perspectiva de xarxa (Fornito i Harrison, 2012). En aquest context, el rol de les metodologies basades en teoria de grafs (p. ex. grau de connectivitat) que permeten caracteritzar alteracions en la integritat de la xarxa i en la topologia dels seus *hubs* és crucial (Bullmore i Sporns, 2009). Addicionalment, el fet que la nostra troballa al putamen ventral repliqués en els estudis de connectivitat total i local reforça la idea que els nodes veïns tendeixen a interaccionar amb major força de connectivitat que els nodes distants. Així doncs, la connectivitat local o modular encobreix la connectivitat distant en aquelles anàlisis en les quals es mesura la connectivitat total (Diez i Sepulcre, 2018). Per tant, si es vol capturar la connectivitat tant local com distant, és recomanable implementar metodologies que segreguin la connectivitat en futurs estudis.

## 6. CONCLUSIONS

A partir dels estudis inclosos en aquesta tesi, hem extret les següents conclusions:

- Els símptomes obsessius-compulsius subclínics totals es relacionen amb alteracions en el circuit CSTC, fet que concorda amb els models neurobiològics del TOC. Tot i

això, les nostres troballes també recolzen la implicació de regions fora d'aquest circuit en el desenvolupament d'aquests símptomes.

- Els símptomes obsessius-compulsius subclínics dimensionals es vinculen a diferents alteracions neurobiològiques. Els símptomes *obsessius* s'associen amb alteracions límbiques. Els símptomes de *dubte-comprovació* es vinculen amb canvis en el circuit CSTC cognitiu ventral, l'ínsula, el FFOI i l'istme del cos callós. En canvi, els símptomes d'*ordre* es relacionen amb canvis en els circuits CSTC sensoriomotor i cognitiu ventral, així com en el còrtex parietal superior, mentre que els símptomes d'*acumulació* es relacionen amb variacions en diferents circuits CSTC, el que indica una alteració més extensa.

- El canvi estructural associat als símptomes d'*ordre* és específic dels nens menors de 10 anys, fet que suggereix que aquest grup demogràfic pot ser especialment vulnerable al desenvolupament de símptomes d'*ordre.* Contràriament, certs canvis estructurals i funcionals vinculats als símptomes d'*acumulació* són específics dels participants de més de 10 anys, especialment en nenes. Per tant, les nenes preadolescents poden presentar una major susceptibilitat a desenvolupar símptomes d'*acumulació.*

- Polimorfismes en els gens GRM7, GNAQ, implicats en la modulació de la transmissió glutamatèrgica, i en el gen PARVA, associat al citoesquelet d'activa, a l'adhesió, motilitat i supervivència cel·lular, predisposen a presentar propietats *attractores* augmentades en l'hipocamp, que es vinculen als símptomes obsessius-compulsius subclínics totals.

- Polimorfismes en els gens ATP1B1 i TESC, que contribueixen al manteniment de gradients electroquímics i al control del pH cel·lular, actuen com a factor de risc i factor protector, respectivament, en l'augment de les propietats *attractores* del còrtex parietal superior, que es vinculen amb els símptomes d'*ordre.*

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# 8. References

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