## Genetic & Environmental risk factors for Obsessive-Compulsive Symptoms: Do they affect the same brain?

den Braber, A.

2012

***document version***
Publisher's PDF, also known as Version of record

# 6 White matter differences in monozygotic twins discordant or concordant for obsessive-compulsive symptoms: A combined Diffusion Tensor Imaging/Voxel-Based Morphometry study

This chapter is published as:
A. den Braber, D. van 't Ent, D.I. Boomsma, D.C. Cath, D.J. Veltman, P.M. Thompson, and E.J.C. de Geus. White matter differences in monozygotic twins discordant or concordant for obsessive-compulsive symptoms: A combined Diffusion Tensor Imaging/Voxel-Based Morphometry study, Biological Psychiatry (2011); 70(10): 969-77.

## Abstract

**Background:** Neuroimaging studies of obsessive-compulsive disorder (OCD) patients point to deficits in cortico-striato-thalamo-cortical circuits that might include changes in white matter. The contribution of environmental and genetic factors to the various OCD-related changes in brain structures remains to be established.
**Methods:** White matter structures were analyzed in 140 subjects with both diffusion tensor imaging and voxel-based morphometry. We studied 20 monozygotic twin pairs discordant for obsessive-compulsive symptoms (OCS) to detect the effects of environmental risk factors for obsessive-compulsive (OC) symptomatology. Furthermore, we compared 28 monozygotic twin pairs concordant for low OCS scores with 23 twin pairs concordant for high OCS scores to detect the effects of genetic risk factors for OC symptomatology.
**Results:** Discordant pair analysis showed that the environmental risk was associated with an increase in dorsolateral-prefrontal white matter. Analysis of concordant pairs showed that the genetic risk was associated with a decrease in inferior frontal white matter. Various white matter tracts showed opposite effects of environmental and genetic risk factors (e.g., right medial frontal, left parietal and right middle temporal) illustrating the need for designs that separate these classes of risk factors.
**Conclusions:** Different white matter regions were affected by environmental and genetic risk factors for OC symptomatology, but both classes of risk factors might, in aggregate, create an imbalance between the indirect loop of the cortico-striato-thalamo-cortical network (to the dorsolateral-prefrontal region) –important for inhibition and switching between behaviors– and the direct loop (to the inferior frontal region), that contributes to the initiation and continuation of behaviors.

## Introduction

Obsessive-compulsive symptoms (OCS) have been defined as recurrent, persistent, and intrusive anxiety provoking thoughts or images (obsessions) and subsequent repetitive behaviors (compulsions) performed to reduce anxiety and/or distress caused by the obsessions (American Psychiatric Association, 1994). When a person has these obsessions and/or performs compulsions for more than one hour a day and these thoughts and rituals significantly interfere with his/her daily life routines, the person fulfills the criteria for obsessive-compulsive disorder (OCD). OCD is generally assessed with structured clinical interviews (e.g., the Structured Clinical Interview for DSM Disorders [SCID]) (American Psychiatric Association, 1994). Additionally, questionnaires such as the Padua Inventory (Sanavio, 1988) and quantitative versions of the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS)

(Goodman et al., 1989a; Goodman et al., 1989b) might be used to rate OCS severity. The life-time prevalence of OCD is 0.5-2% (American Psychiatric Association, 1994; Grabe et al., 2000), but obsessions are much more prevalent in the general population – as high as 72% (Rachman and de Silva, 1978), and the prevalence of OCS reaches 20% (Fullana et al., 2009).

Over the last two decades, neuroimaging studies have indicated several neurobiological changes underlying the psychological and behavioral deficits of OCD. Structural magnetic resonance imaging (MRI) has revealed regional volume differences in the ventral prefrontal cortex, dorsolateral prefrontal cortex (DLPFC), basal ganglia, anterior cingulate cortex, parietal cortex and thalamus (Menzies et al., 2007; Pujol et al., 2004; Radua and Mataix-Cols, 2009; Radua et al., 2010; Rotge et al., 2009; Valente Jr. et al., 2005; van den Heuvel et al., 2009). Findings from functional neuroimaging studies are largely consistent with structural MRI findings and show altered regional activation in the aforementioned brain structures during performance of cognitive tasks and after symptom provocation (for a review, see Menzies et al. (2008a)). Together, these findings contributed to the widely accepted neuroanatomical model of OCD involving the direct and indirect cortico-striato-thalamo-cortical (CSTC) loops (Mataix-Cols and van den Heuvel, 2006; Saxena and Rauch, 2000). The direct loop functions as a self-reinforcing feedback loop that contributes to the initiation and continuation of behaviors. The indirect loop functions as a negative feedback loop important for inhibiting and switching between behaviors (Mataix-Cols and van den Heuvel, 2006). It has been hypothesized that an imbalance between these loops, resulting in a hyperactive ventral and hypoactive dorsal frontal-striatal system, might mediate obsessive-compulsive (OC) symptomatology (Mataix-Cols and van den Heuvel, 2006; Saxena and Rauch, 2000). Although a disturbance in these CSTC loops seems to be the neurological basis for OCD, several imaging studies suggest the involvement of other brain regions in OCD as well. For example, Menzies et al. (2008) recently proposed an extended model that includes various brain areas (e.g., anterior cingulate cortex, amygdala, parietal areas) that are functionally connected to the ventral and dorsal CSTC loops.

So far, anatomical models of OCD have been mainly based on structural and functional MRI analyses that focused on gray matter differences in OCD patients compared with control subjects. More recent studies suggest an additional role for white matter abnormalities in the etiology of OCD (Cannistraro et al., 2006; Garibotto et al., 2010; Ha et al., 2009; Menzies et al., 2008b; Nakamae et al., 2008; Saito et al., 2008; Szeszko et al., 2005; Yoo et al., 2007; Nakamae et al., 2011), possibly related to variation in genes involved in oligodendrocyte development (Stewart et al., 2007b). Diffusion tensor imaging (DTI) can be used to study white matter integrity, for instance in tracts that interconnect the brain regions of

the CSTC loops. DTI provides a measure of diffusion of water molecules within tissues, permitting the investigation of brain tissue microstructure. In structures with a highly coherent directional organization, (e.g., white matter tracts in the brain), the dominant direction of diffusion is parallel to the fiber direction, so that diffusion becomes more anisotropic (Basser and Pierpaoli, 1996; Beaulieu, 2002; Le Bihan et al., 2001; Mori and Zhang, 2006). Fractional anisotropy (FA), a value that can be derived from diffusion tensor images, describes the degree of anisotropy within a voxel. Reduced FA might be interpreted as a reduced density of fibers, less directional coherence of fibers, or a reduced degree of myelination of fibers, all suggesting damaged or disorganized or under-developed white matter (Basser and Pierpaoli, 1996; Beaulieu, 2002; Le Bihan et al., 2001; Mori and Zhang, 2006). To further investigate the nature of white matter alterations, T1-weighted scans can be analyzed with voxel-based morphometry (VBM). VBM, performed on white matter segmentations provides information on regional white matter volume differences (Ashburner and Friston, 2000; Ashburner and Friston, 2001). If an increase in FA is accompanied by an increase in white matter volume, the higher white matter integrity might indicate fibers that are more dense or more myelinated.

A few studies that used DTI to measure white matter abnormalities in OCD patients compared with healthy control subjects (Cannistraro et al., 2006; Garibotto et al., 2010; Ha et al., 2009; Menzies et al., 2008b; Nakamae et al., 2008; Saito et al., 2008; Szeszko et al., 2005; Yoo et al., 2007; Nakamae et al., 2011) have reported white matter differences near the caudate nucleus and thalamus (Cannistraro et al., 2006; Yoo et al., 2007), whereas others found differences predominantly in medial frontal and parietal regions (Ha et al., 2009; Menzies et al., 2008b; Szeszko et al., 2005). In addition, directly conflicting results were found, with reports of lower (Garibotto et al., 2010; Ha et al., 2009; Szeszko et al., 2005) and higher (Cannistraro et al., 2006) FA in the left cingulate, or no differences between patients and control subjects in this region at all (Menzies et al., 2008b). Such inconsistencies are usually explained as being due to methodological differences between studies, such as heterogeneity of patient groups and differences in sample size, scanning modalities/parameters, and analysis methods. However, there might also be 'true' variability in the underlying neurobiology of OCD. That is, different white matter tract abnormalities might lead to comparable behavioral changes, because they occur in parts of the same brain network that regulates anxiety and safety behaviors. Such heterogeneity in affected white matter fibers might, in turn, reflect the differential influence of environmental and genetic risk factors for OC symptomatology that might impact different parts of the brain (den Braber et al., 2010).

Most clinical DTI studies employ standard case-control designs, comparing healthy control subjects with a group of affected individuals. These studies, however, cannot disentangle whether differences in brain white matter integrity are due to environmental versus genetic risk factors. One design that makes a distinction between environmentally and genetically mediated neurobiological differences that underlie the development of behavioral traits such as OCD is the discordant/concordant monozygotic (MZ) twin design (de Geus et al., 2007; den Braber et al., 2010; van 't Ent et al., 2009; Wolfensberger et al., 2008). Nearly all MZ twins begin life with identical genomes, so behavioral discordances are likely to arise from differential exposure to environmental influences that can differentially modify gene expression in subjects with identical genotypes (Heijmans et al., 2009). Consequently, differences in central nervous system white matter between the high-risk twin and the low-risk co-twin reflect environmental effects on the brain, rather than effects of genetic variation. To detect the effects of genetic risk factors, neuroimaging results can be compared between MZ twins who both score high for measures of OCS and MZ twins who both score very low for OCS. These MZ concordant-high- and low-scoring twins are likely to come from families with either high or low vulnerability for OCD. Genetic epidemiological studies that compare MZ and dizygotic twin resemblance in OCS have already shown that the shared family environment does not influence these symptoms and that the familial vulnerability for this trait translates entirely to genetic vulnerability (Clifford et al., 1984; Jonnal et al., 2000; van Grootheest et al., 2007). Therefore, a comparison between MZ twins who both score high (concordant-high) for measures of OCS and MZ twins who both score low (concordant-low) for OCS can reveal white matter differences due to influences of genetic risk factors.

Our study aimed to examine the differential impact of non-shared environmental versus genetic influences on white matter regions in subjects at high risk for OCD. We compared DTI-derived FA maps between twins scoring low and twins scoring high on OCS from discordant MZ pairs and between concordant pairs where both twins scored either low or high on OCS. To confirm that an increase (decrease) in FA was accompanied by an increase (decrease) in white matter volume, we additionally examined white matter volumes in these specific regions with VBM.

## Methods and materials

### Participants

The twin pairs included in this study were recruited from the Netherlands Twin Register (Boomsma et al., 2006). Surveys were sent to twin families including the Padua Inventory Abbreviated (PI-R-ABBR) (Cath et al., 2008; van Oppen et al., 1995). Completed PI-R-ABBR questionnaires were returned by 815 MZ twin pairs

**Table 6.1. Twin sample demographics**

| | | Twin pairs | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Discordant (environmental risk measure) | | | | Concordant (genetic risk measure) | | | |
| | | high (n=20) | low (n=20) | | | high (n=46) | low (n=56) | | |
| Demographic data | Female | 14 | | | | 17 | 20 | | |
| | Male | 6 | | | | 6 | 8 | | |
| | Age (years (SD)) | 35.60 (8.68) | | | | 36.00 (10.55) | 37.50 (8.79) | | |
| | | mean (±SD) | mean (±SD) | t-value | *p* | mean (±SD) | mean (±SD) | t-value | *p* |
| Obsessive-compulsive symptoms | PI-R-ABBR (0-48) | 20.07 (5.03) | 4.73 (1.84) | 14.51 | <0.001 | 20.42 (4.56) | 4.18 (2.19) | 22.31 | <0.001 |
| | Y-BOCS severity lifetime (0-40) | 7.70 (5.69) | 6.70 (8.18) | 0.64 | .527 | 10.41 (7.15) | 3.18 (4.54) | 5.13 | <0.001 |
| | Y-BOCS severity current (0-40) | 5.45 (5.62) | 1.45 (2.19) | 3.64 | 0.001 | 7.54 (5.83) | 0.95 (2.13) | 6.95 | <0.001 |
| Comorbidity | MINI : | | | | | | | | |
| | Depression (n) | 2 | 0 | | | 0 | 0 | | |
| | Panic disorder (n) | 1 | 0 | | | 0 | 0 | | |
| | Agoraphobia (n) | 2 | 0 | | | 0 | 0 | | |
| | Social disorder (n) | 1 | 0 | | | 2 | 0 | | |
| | Post-traumatic stress disorder (n) | 1 | 0 | | | 0 | 0 | | |
| | Generalized anxiety disorder (n) | 3 | 0 | | | 7 | 0 | | |
| | Tic (0-8) | 0.40 (0.75) | 0.20 (0.41) | 1.25 | 0.214 | 0.30 (0.66) | 0.09 (0.29) | 2.03 | 0.046 |
| | BDI (0-39) | 4.65 (7.50) | 3.05 (2.80) | 1.73 | 0.089 | 3.50 (3.17) | 1.38 (2.18) | 2.47 | 0.016 |
| | STAI (0-60) | 13.85 (8.54) | 12.25 (6.13) | 0.83 | 0.409 | 13.37 (7.39) | 8.55 (7.36) | 2.91 | 0.005 |
| | STAS (0-30) | 0.20 (0.70) | 0.00 (0.00) | 0.91 | 0.365 | 0.46 (2.09) | 0.11 (0.49) | 1.09 | 0.282 |

*Values are given as (n), unless otherwise indicated. Twin pairs: number of female and male twin pairs; age: age at time of magnetic resonance imaging (MRI) examination (in years). PI-R-ABBR: mean Padua Inventory Abbreviated Scores (SD); Y–BOCS severity lifetime/current: mean Yale–Brown Obsessive–Compulsive Scale severity scores (SD) across whole life span and at the time of MRI; MINI (depression, panic disorder, agoraphobia, social disorder, posttraumatic stress disorder, generalized anxiety disorder): number of subjects with current comorbid disorder (measured with the Mini International Neuropsychiatric Interview); Tic: mean Tic scores (SD) at time of MRI; BDI: mean Beck Depression Inventory scores (SD) at time of MRI; STAI: mean State Trait Anxiety Inventory scores (SD) at time of MRI; STAS: mean State Trait Anger Scale scores (SD) at time of MRI.*

(222 male; 593 female). From this sample we selected twin pairs in the age range between 18 and 60 years that scored discordant, concordant-high or concordant-low for OCS. A twin pair was classified as discordant for OCS if one twin scored OCS high (>16) and the co-twin scored OCS low (≤7). A twin pair was classified as concordant-high for OCS if both twins scored ≥15, with at least one twin scoring ≥16. A twin pair was classified as concordant-low for OCS if both twins scored ≤7. These PI-R-ABBR cut-off scores were derived from sensitivity and specificity measurements in a sample of OCD patients when compared with clinical control subjects (n=120; mean scores 20.7, SD 8.1; sensitivity 0.74 and specificity 0.72 at the best cut-off point of 16 (Cath et al., 2008)). For more details on sample selection we refer to den Braber et al. (2010). A final sample of 71 MZ twin pairs participated in this MRI study, including 20 discordant, 23 concordant-high and 28 concordant-low twin pairs **(table 6.1)**. The MRI protocol could not be completed by two subjects (metal artifact, panic attack). In the final sample (n=140), 2 twins with high OCS scores from the discordant group and 5 twins with high OCS scores from the concordant-high group met clinical diagnosis for OCD. Furthermore, 3 twins with high OCS scores and 1 twin with a low OCS score from the discordant group and 6 twins from the concordant-high group used selective serotonin reuptake inhibitors (SSRIs).

### Protocol

Participants were administered diagnostic interviews and questionnaires, including questions on demography, life-events, comorbidity, type and severity of OCS, tics, state-anger, and state anxiety (for a detailed description of the administered diagnostic interviews and questionnaires, please refer to den Braber et al. (2010)). All twins were asked to collect mucosal cell samples for DNA extraction to test zygosity. The ethical review board of the VU University medical centre approved the study. All participants provided written informed consent.

### Image acquisition

The MRI session consisted of an anatomical scan of approximately 6 minutes and a DTI scan of approximately 3 minutes. During the scan sessions, the twins remained inside the scanner and were asked to minimize head movement during and between consecutive runs. To reduce motion artifacts, the head of each participant was immobilized with foam pads.

MRI was performed on a 3.0 T Intera MR system (Philips, Medical Systems, Best, The Netherlands) with a standard SENSE receiver head coil. The anatomical scan consisted of 182 coronal slices with a 3-dimensional T1-weighted gradient-echo sequence (flip angle 8°; Repetition Time = 9.69 ms; Echo Time = 4.60 ms; matrix, 256x256 pixels; voxel size, 1.00x1.00x1.20 mm). Diffusion tensor images were obtained in 32 directions by using single-shot echoplanar acquisition (flip angle

90º; Repetition Time = 4834 ms; Echo Time = 94 ms; matrix, 112x110 pixels; voxel size, 2.00x2.00x3.00 mm; b-value 1000 s/mm2, 38 axial slices).

### Data analysis

FA maps were calculated from raw DTI scans with the Medical Image Navigation and Research Tool by INRIA (MEDINRIA, Asclepios Research Project - INRIA Sophia Antipolis, France). MRI data were further analyzed with SPM8 (Wellcome Department of Imaging Neuroscience, London, United Kingdom). T1-weighted magnetic resonance images were segmented into gray matter, white matter and cerebrospinal fluid, and normalized to a group template (i.e., a specific template for the discordant and concordant twins) with the Diffeomorphic Anatomical Registration Through Exponential Lie algebra (DARTEL) algorithm, and subsequently warped from DARTEL space to the standard Montreal Neurological Institute (MNI) brain. FA maps were first co-registered with T1-weighted magnetic resonance images and normalized with each subject's T1 to DARTEL to MNI warp parameters. Subsequently, data were spatially smoothed with an 8 mm isotropic Gaussian kernel.

### Statistical tests

Differences in survey- and interview-based variables were tested with a mixed-model analysis of variance (Mixed Models Linear menu item in SPSS; SPSS, Chicago, Illinois) with twin pair type (discordant vs. concordant) and OCS score level (high vs. low) as two fixed factors and family as a random factor to account for within-twin pair dependence. Statistical results were considered significant at $p < 0.05$, Bonferroni corrected.

Differences in FA maps between OCS high- and OCS low-scoring twins from discordant pairs were investigated with a paired sample t-test. Differences in FA maps between concordant OCS high and concordant OCS low twin pairs were assessed with an analysis of variance group comparison. To account for within-twin pair correlations, FA maps of the twin and co-twin of each concordant pair were entered as repeated measures. Group differences are reported at an uncorrected individual voxel threshold of $p < 0.005$, with a minimal cluster extent of 50 voxels, which is slightly more conservative as used in previous DTI studies of OCD (Cannistraro et al., 2006; Garibotto et al., 2010; Szeszko et al., 2005).

To test whether an increase (decrease) in FA is accompanied by an increase (decrease) in white matter volume, an additional region of interest (ROI) analysis was performed on the structural VBM data. That is, we tested for increased (decreased) white matter volumes in the discordant and concordant structural data specifically in spherical ROIs (radius 10 mm) centered on the coordinates where discordant and concordant pairs showed maximally increased (decreased)

FA. For these post hoc ROI analyses, an individual voxel p-value threshold of $p<0.05$ was applied, corrected for multiple comparisons (Family Wise Error).

## Results

### Questionnaire and interview data

As expected, OCS high- compared with low-scoring twins in both the discordant and concordant groups showed significant higher scores on the PI-R-ABBR, and current Y-BOCS severity scale **(table 6.1)**. In addition, high-scoring twins were more often diagnosed with current comorbid disorders, which were absent in the low-scoring twins.

### Environmental risk: OCS high- versus low-scoring twins from discordant pairs

#### Diffusion tensor imaging – fractional anisotropy

**Table 6.2A** and **figure 6.1** summarize the FA results of the OCS high versus low within twin pair comparison of the discordant pairs. Relative to their low-scoring co-twins, OCS high-scoring twins exhibited clusters of increased FA in right orbitofrontal (cluster label A in **table 6.2A** and **figure 6.1**), left dorsolateral prefrontal (cluster label B), left precentral (cluster label C), left corpus callosum (cluster label D), left cingulate (cluster label E), left insula (cluster label F), right superior parietal (cluster label G), and right temporal (cluster label H) regions and bilaterally in cerebellar regions (cluster labels I and J). Clusters of decreased FA in OCS high compared with their low-scoring co-twins were observed bilaterally in medial frontal and temporal regions (cluster labels K, L, N, P and Q in **table 6.2A** and **figure 6.1**), right insula (cluster label M), left parietal (cluster label O), and right occipital (cluster label R) regions and left brainstem/pons (cluster label S).

#### Structural VBM data – region of interest analysis

ROI analysis of the structural VBM data at coordinates that showed maximal within pair differences in FA in the discordant sample revealed a significant volume increase in left dorsolateral prefrontal white matter. Furthermore, a trend towards decreased white matter volumes in the high- compared with low-scoring twins was found in right medial frontal and left parietal regions (**table 6.2B** and indicated z-scores in **figure 6.1**).

### Genetic risk: concordant-high- versus concordant-low-scoring twins

#### Diffusion tensor imaging – fractional anisotropy

**Table 6.3A** and **figure 6.2** show clusters of OCS related FA differences between the concordant-high and low twin pairs. Concordant-high-scoring twins compared with OCS low-scoring twins exhibited clusters of increased FA in right medial frontal (cluster label T in **table 6.3A** and **figure 6.2**) and right temporal (cluster

## Table 6.2. Environmental Risk: Regional White Matter Differences Between OCS High and Low Twins of the Discordant Sample

| Regional White Matter FA Within-Pair Differences[a] | | | | | | | | | Regional White Matter Volume Within-Pair Differences[b] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Test | MNI coordinates | | | Z score | p-value | voxels (*n*) | Anatomical location | Cluster label | MNI coordinates (ROI VBM) | | | T-value | p-value | voxels (*n*) |
| | x | y | z | | | | | | x | y | z | | | |
| high>low | 18 | 39 | -23 | 3.6 | <0.001 | 98 | right orbitofrontal | A | – | – | – | – | – | – |
| | -34 | 15 | 31 | 3.19 | 0.001 | 57 | left dorsolateral prefrontal | B | -25 | 14 | 31 | 4.94 | 0.008[c] | 194 |
| | -25 | -27 | 66 | 3.46 | <0.001 | 65 | left precentral | C | – | – | – | – | – | – |
| | -13 | -10 | 27 | 3.63 | <0.001 | 189 | left corpus callosum | D | – | – | – | – | – | – |
| | -13 | -7 | 63 | 3.31 | <0.001 | 81 | left cingulate | E | – | – | – | – | – | – |
| | -41 | -30 | 21 | 3.37 | <0.001 | 101 | left insula | F | – | – | – | – | – | – |
| | 29 | -51 | 57 | 3.16 | 0.001 | 69 | right superior parietal | G | – | – | – | – | – | – |
| | 48 | -16 | -26 | 3.08 | 0.001 | 50 | right temporal | H | – | – | – | – | – | – |
| | -17 | -73 | -39 | 3.34 | <0.001 | 295 | left cerebellum, pyramis/tonsil | I | – | – | – | – | – | – |
| | 20 | -70 | -35 | 3.67 | <0.001 | 488 | right cerebellum, uvula/piramis | J | – | – | – | – | – | – |
| high<low | 11 | -15 | 63 | 4.23 | <0.001 | 167 | right medial frontal | K | 11 | -18 | 68 | 3.63 | 0.075[d] | 88 |
| | 27 | -15 | 36 | 3.6 | <0.001 | 551 | right medial frontal | L | – | – | – | – | – | – |
| | 32 | 2 | 15 | 3.36 | <0.001 | | right insula | M | – | – | – | – | – | – |
| | -25 | 15 | 24 | 3.12 | 0.001 | 128 | left medial frontal | N | – | – | – | – | – | – |
| | -33 | -67 | 32 | 3.35 | <0.001 | 50 | left parietal | O | -28 | -61 | 26 | 3.59 | 0.081[d] | 33 |
| | -28 | -67 | 12 | 3.24 | 0.001 | 53 | left temporal | P | – | – | – | – | – | – |
| | 35 | -6 | -30 | 4.08 | <0.001 | 128 | right middle temporal | Q | – | – | – | – | – | – |
| | 15 | -91 | 17 | 3.92 | <0.001 | 91 | right occipital | R | – | – | – | – | – | – |
| | -9 | -12 | -27 | 3.27 | 0.001 | 64 | left brainstem, pons | S | – | – | – | – | – | – |

*MNI, Montreal Neurological Institute; VBM, voxel-based morphometry; other abbreviations as in table 6.1.*

***a** Clusters with regional fractional anisotropy (FA) differences between OCS high and low twins in the discordant sample. Test: test for significant FA increases (high > low) or decreases (high < low) in OCS high- relative to OCS low-scoring twins; MNI coordinates (mm): location of voxel with largest effect size; Z score: z value of voxel with largest effect size; p-value: cluster p-value; Voxels (n): number of voxels in cluster; cluster label: alphabetical cluster label as displayed in anatomical overlays of figure 6.1.* **b** *Clusters with regional white matter volume differences between OCS high and low twins in the discordant sample in spherical region of interest (ROIs) (radius 10mm) centered on the coordinates where discordant pairs showed maximally increased (decreased) FA. MNI coordinates (mm): location of voxel with largest effect size; p-value: cluster p-value family-wise error (FWE)-corrected; voxels (n): number of voxels in cluster.* **c** *$p < .05$, FWE-corrected.* **d** *Trend toward $p < .05$, FWE-corrected.*

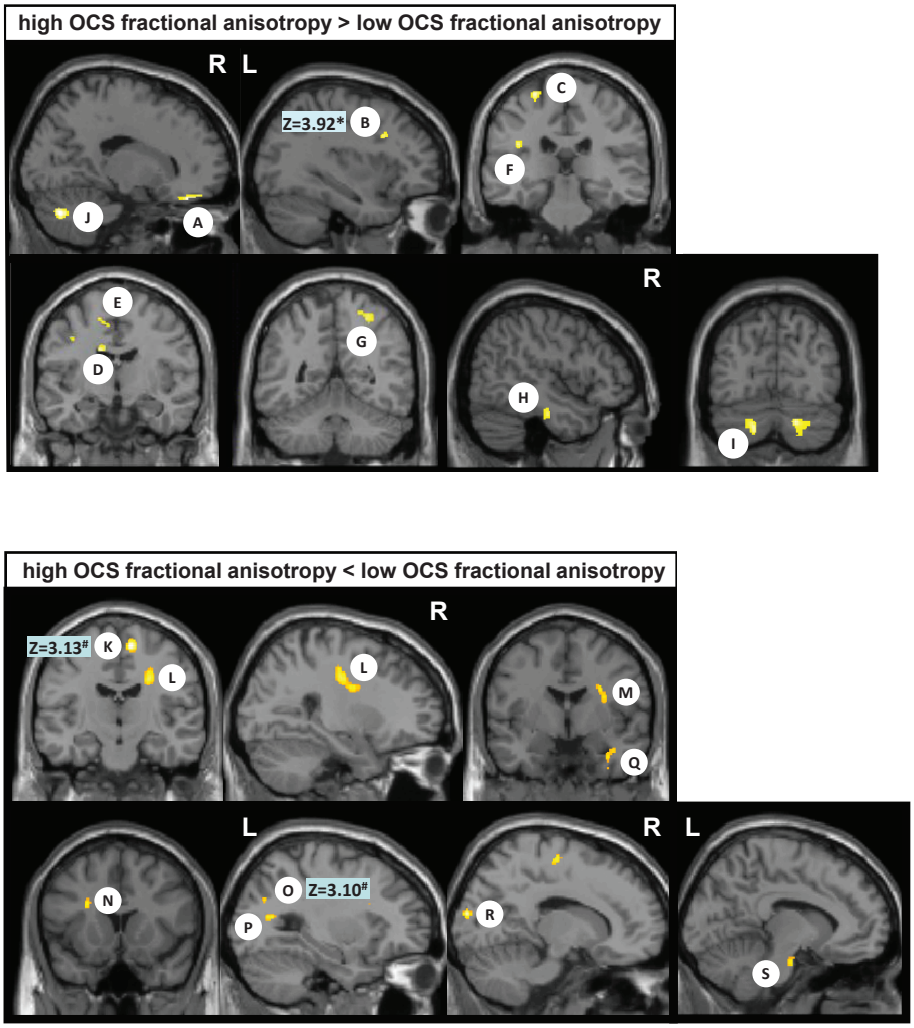


***Figure 6.1.*** *Environmental risk: fractional anisotropy (FA) in high obsessive-compulsive symptoms (OCS) relative to low OCS discordant twins. Brain regions showing increased (top panels: high > low) and reduced (bottom panels: high < low) FA in OCS high vs. low twins of the discordant sample. Z: z value of voxel with largest effect size derived from additional voxel-based morphometry–region-of-interest analysis, *p < .05, family-wise error (FWE)-corrected; #trend toward p < .05, FWE-corrected.*

label W) regions and bilaterally in parietal regions (cluster label U and V). A cluster of decreased FA in OCS high- compared with low-scoring twins was observed in the left inferior frontal lobe (cluster label X in **table 6.3A** and **figure 6.2**).

### Structural VBM data - region of interest analysis

ROI analysis of the structural VBM data at coordinates that showed maximal between-group differences in FA in the concordant sample revealed a significant decrease in white matter volume in the high compared with low concordant twins

in the left inferior frontal lobe. No clusters of increased white matter volumes were found (**table 6.3B** and indicated z-scores in **figure 6.2**).

Because this study included subjects with SSRI medication we conducted additional analyses to test whether removing these subjects from the analyses would affect the results. We re-ran the analysis on 17 discordant pairs and 20 concordant-high versus 28 concordant-low pairs not taking SSRIs. These analyses did not affect the pattern of results.

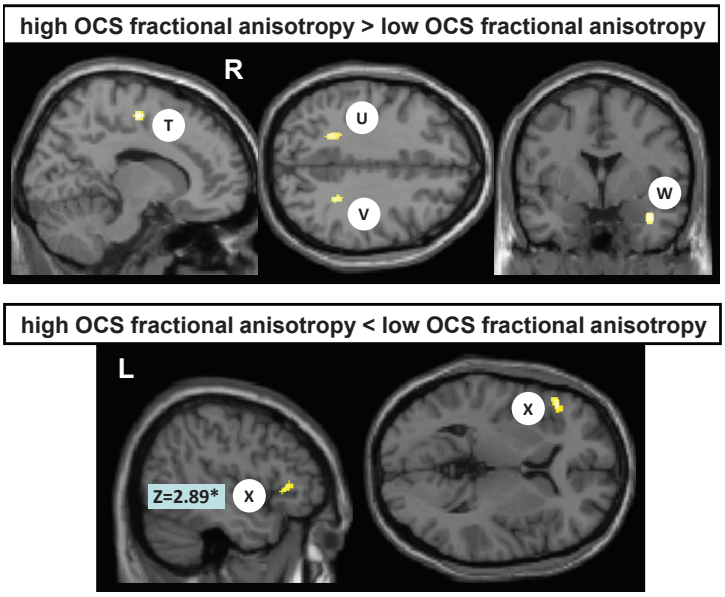


***Figure 6.2.*** *Genetic risk: FA in high OCS relative to low OCS concordant twins. Brain regions showing increased (top panels: high > low) and reduced (bottom panels: high < low) FA in concordant-high vs. concordant-low twins. Z: z value of voxel with largest effect size derived from additional voxel-based morphometry–region-of-interest analysis, *p < .05, FWE-corrected. Abbreviations as in figure 6.1.*

## Table 6.3. Genetic Risk: Regional White Matter Differences Between OCS High and Low Twins of the Concordant Sample

| Regional White Matter FA Between-Group Differences[a] | | | | | | | | | Regional White Matter Volume Between-Group Differences[b] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Test | MNI coordinates | | | Z score | p-value | voxels (*n*) | Anatomical location | Cluster label | MNI coordinates (ROI VBM) | | | T-value | p-value | voxels (*n*) |
| | x | y | z | | | | | | x | y | z | | | |
| high>low | 14 | -13 | 56 | 3.41 | <0.001 | 78 | right medial frontal | T | – | – | – | – | – | – |
| | -21 | -48 | 38 | 3.22 | 0.001 | 142 | left parietal | U | – | – | – | – | – | – |
| | 29 | -43 | 36 | 3.08 | 0.001 | 51 | right parietal | V | – | – | – | – | – | – |
| | 39 | 0 | -24 | 3.40 | <0.001 | 86 | right middle temporal | W | – | – | – | – | – | – |
| high<low | -51 | 27 | 3 | 3.99 | <0.001 | 166 | left inferior frontal | X | -46 | 27 | 1 | 3.03 | 0.047[c] | 41 |

***a*** *Cluster with regional FA differences between concordant-high and low twins. Test: test for significant FA increases (high>low) or decreases (high<low) in OCS high- relative to OCS low-scoring twins; MNI coordinates (mm): location of voxel with largest effect size; Z score: z value of voxel with largest effect size; p-value: cluster p-value; voxels (n): number of voxels in cluster; cluster label: alphabetical cluster label as displayed in anatomical overlays of figure 6.2.*

***b*** *Clusters with regional white matter volume differences between OCS high and low twins in the concordant sample in spherical ROIs (radius 10 mm) centered on the coordinates where concordant pairs showed maximally increased (decreased) FA. MNI coordinates (mm): location of voxel with largest effect size; p-value: cluster p-value FWE-corrected; voxels (n): number of voxels in cluster. Abbreviations as in table 6.2.*

***c*** *$p < .05$, FWE-corrected.*

## Discussion

White matter structures were compared with a combined DTI-VBM analysis within MZ twin pairs discordant for OCS and between MZ twin pairs concordant-low or concordant-high for OCS. Discordant pair analysis indicated that environmental risk factors for OC symptomatology were associated with increases in dorsolateral prefrontal white matter. Concordant pairs analysis suggested that genetic risk factors for OC symptomatology were associated with decreases in inferior frontal white matter. Remarkably, DTI analysis indicated that some white matter tracts show FA alterations that are in the opposite direction in subjects at high environmental risk compared with subjects at high genetic risk (e.g., right medial frontal, left parietal and right middle temporal). Results are discussed in more detail in the following text, in which we focus on the areas that were detected by DTI and confirmed by the VBM analysis. VBM not only shows that the white matter differences identified by DTI indicate a change in the number of fibers or higher degree of myelination of fibers, it also provides a within-study replication of white matter abnormalities by a different method.

### Environmentally mediated white matter alterations

The dorsolateral prefrontal region showed increased white matter integrity (FA), accompanied by increased white matter volume (VBM), in OCS high- compared with OCS low-scoring twins only in the discordant sample. Thus, these white matter differences are likely related to environmental risk factors for OC symptomatology. An increased FA in the dorsolateral prefrontal region, as found in subjects at high environmental risk for OCD, was also found by Ha et al. (2009) and is in line with literature in OCD (Menzies et al., 2008a). The DLPFC has been related to executive processing, including attention, response inhibition, cognitive planning, and decision making (Faw, 2003; Newman et al., 2003; Ridderinkhof et al., 2004). Neuropsychological studies have typically associated dysfunction of the DLPFC with perseverative, disinhibited behaviors, which OCD patients particularly show during the completion of their compulsions (Friedlander and Desrocher, 2006). The finding of systematic differences in white matter integrity in the DLPFC is consistent with the commonly accepted neurobiological model of CSTC abnormalities in OCD (Graybiel and Rauch, 2000; Insel and Winslow, 1992). In addition, this region was also implicated in OCD by a previous functional MRI study by our group that showed an environmentally mediated decrease in DLPFC activity during the performance of a Tower of London planning paradigm (den Braber et al., 2010).

Some white matter regions showed altered FA in high compared with low discordant twins that were not corroborated by our VBM-ROI analysis, but replicate previous DTI findings in OCD patients. These include the corpus callosum and

the cingulate bundle (Cannistraro et al., 2006; Garibotto et al., 2010; Yoo et al., 2007). An environmentally mediated increase in FA was found in the body of the corpus callosum, which interconnects motor and posterior parietal regions (Saito et al., 2008). This is in line with results from a morphological study that found significantly larger corpus callosal areas in pediatric OCD patients compared with control subjects (Rosenberg et al., 1997). The cingulate bundle is one of the main white matter tracts that connects the gray matter nodes of the neural circuitry implicated in OCD (Locke et al., 1961; Yakovlev and Locke, 1961). We found FA was higher in the cingulate bundle in the discordant OCS high twins, which replicates the finding by Cannistraro et al. (2006) in patients with OCD compared with healthy control subjects. The cingulate effect might be specific to environmental risk factors, because Menzies et al. (2008b), who performed a ROI analysis in OCD patients and their first degree relatives, did not find FA alterations in the cingulate bundle in either patients or their first-degree relatives.

### Genetically mediated white matter alterations

The inferior frontal region showed lower white matter integrity (FA), accompanied by decreased white matter volume (VBM) in OCS high- compared with OCS low-scoring twins only in the concordant sample. These white matter deficits are likely related to genetic risk factors for OC symptomatology. The inferior frontal region is also implicated in the widely accepted neuroanatomical CSTC model for OCD and is involved in a wide range of cognitive processes, including task switching, reversal learning, and cognitive and emotional inhibition (Dillon and Pizzagalli, 2007; Ramnani and Owen, 2004). Furthermore, this region is involved in regulating socially appropriate behaviors, and when impaired a person might show impulsive and disinhibited behavior (Friedlander and Desrocher, 2006). In addition, this region was also implicated in a previous functional study by our group that showed a genetically mediated increase in inferior frontal activity during the performance of a Tower of London planning paradigm (den Braber et al., 2010).

### White matter changes related to both environmental and genetic risk factors

Regions that showed FA alterations in both the discordant and concordant sample include medial frontal, parietal, and temporal regions. Interestingly, if we examine our results in more detail, roughly the same white matter regions show FA alterations, but these are in the opposite direction (increased vs. decreased) in subjects at high environmental risk compared with subjects at high genetic risk (e.g., right medial frontal: reduced FA in discordants, increased FA in concordants; left parietal: reduced FA in discordants, increased FA in concordants; right temporal: reduced FA in discordants, increased FA in concordants). This pattern of opposite DTI findings for the same anatomical region echoes a similar disparity in the extant literature. For instance, some studies found decreased FA in

the corpus callosum in patients with OCD compared with control subjects (Garibotto et al., 2010; Saito et al., 2008), whereas others reported increased FA in OCD patients for this region (Yoo et al., 2007). This pattern of findings makes most sense when we allow environmental and genetic risk factors to affect the brain in different ways. This is, in fact, a major rationale to apply the discordant/concordant twin design.

Patient samples represent an unknown mixture of individuals who developed OCD due to a genetic predisposition and/or environmental triggers. Our results illustrate that, if the study sample predominantly consists of patients with a familial predisposition, findings might differ from those of a study of patients who might have developed the disorder due to a negative environmental experience (e.g., divorce, sexual assault). A previous study attempting to identify genetic markers for OCD by comparing FA alterations in OCD patients, their first-degree relatives, and control subjects found an FA increase in right medial frontal white matter (Menzies et al., 2008b). This result is in line with our finding of a genetically mediated FA increase in the same region, which might indicate that this alteration is specific to individuals at increased genetic risk for OCD. However, this finding could be easily missed if a sample represents a mixture of subjects at increased genetic risk and subjects with environmentally mediated OCD. This might be true of the various studies that failed to find FA alterations in this region (Cannistraro et al., 2006; Garibotto et al., 2010; Yoo et al., 2007). Medial frontal, parietal, and temporal regions have been implicated in the neuroanatomical model for OCD, predominantly through their functional connections with the ventral prefrontal cortex and DLPFC. An alteration in these functional connections may lead to an imbalance between the direct and indirect pathways of the ventral and dorsal frontal-sriatal loops, which subsequently may induce OC-like behavior.

To summarize, inconsistencies between results of previously performed imaging studies might be related, at least in part, to 'true' variability in the underlying neurobiology of OCD. The present results suggest that the effects on central nervous system white matter regions of environmental risk factors for OC symptomatology are distinct from those of the genetic risk for OC symptomatology. These findings are in line with results from a previous functional imaging study by our group (den Braber et al., 2010) in which these same regions were found to be differentially affected by environmental and genetic risk factors for OC symptomatology. Interestingly, when the DTI-VBM and functional MRI results are taken together, they point toward an inverse relation between task-related activity and white matter integrity in OCS (e.g., environmentally mediated decrease in DLPFC activity coupled with higher dorsolateral prefrontal white matter integrity, and genetically mediated increase in inferior frontal activity together with lower inferior frontal white matter integrity). This inverse relation

might be the result of an increase or decrease, respectively, in inhibitory signaling in these specific regions that depends on white matter integrity.

Although different regions seemed to be affected by environmental and genetic risk factors for OC symptomatology, both classes of risk factors strikingly converge on the CSTC loops. Neurobiological changes in OCS induced by environmental risk factors involve the indirect loop of the dorsal CSTC circuit (dorsolateral prefrontal region), which functions as a negative feedback loop important for the inhibition of and switching between behaviors. By contrast, neurobiological changes in OCS induced by genetic risk factors, involve the direct loop of the ventral CSTC circuit (inferior frontal region), which functions as a self-reinforcing feedback loop that contributes to the initiation and continuation of behaviors **(figure 6.3)**.

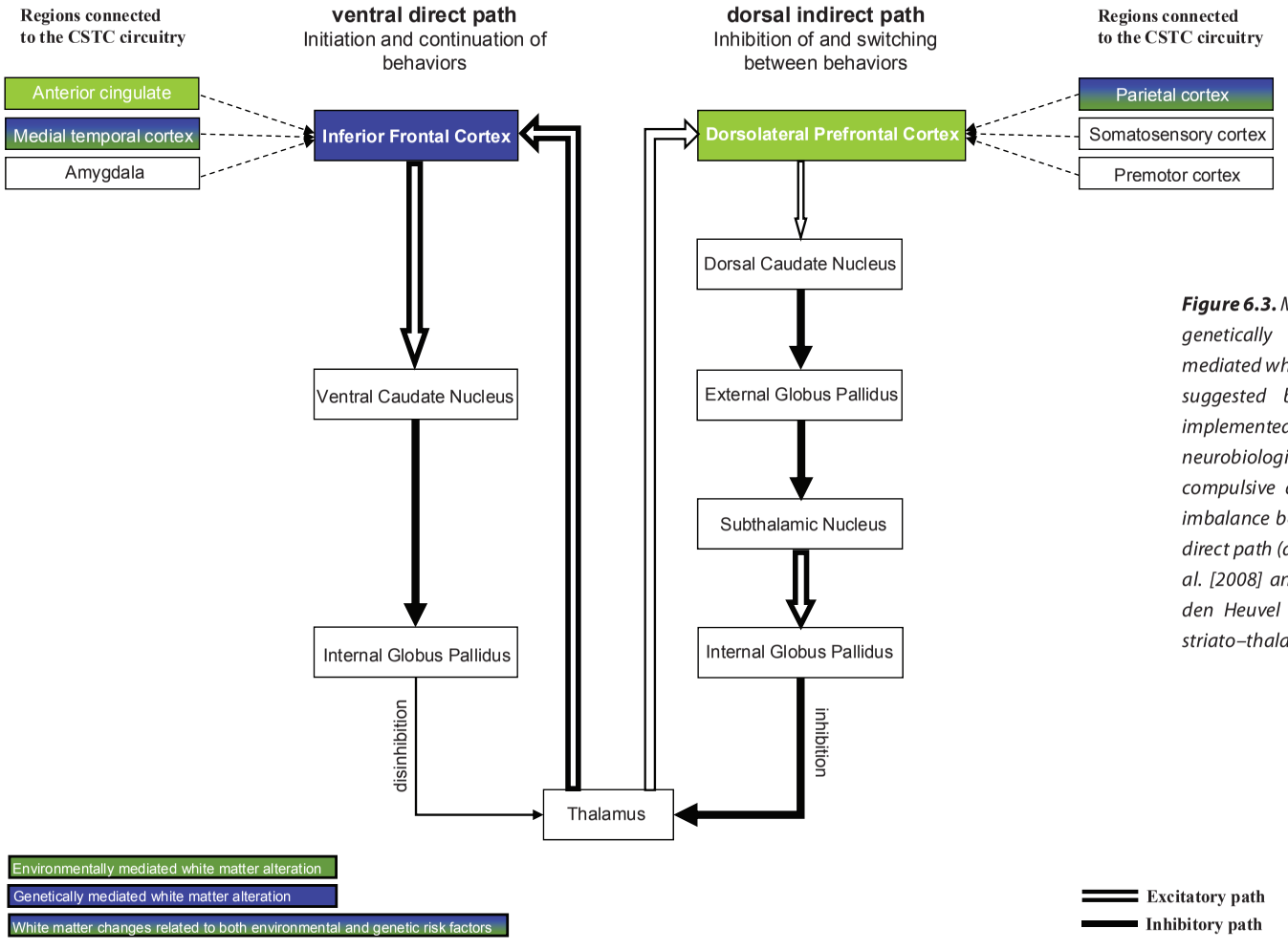


***Figure 6.3.*** *Model illustrating distinct genetically and environmentally mediated white matter alterations as suggested by the present study implemented in the widely accepted neurobiological model for obsessive-compulsive disorder, suggesting an imbalance between the indirect and direct path (adapted from Menzies et al. [2008] and Mataix-Cols and van den Heuvel [2006]). CSTC, cortico–striato–thalamo–cortical.*