

TITLE PAGE

White Matter Microstructure and its Relation to Clinical Features
of Obsessive-Compulsive Disorder:
Findings from the ENIGMA OCD Working Group

Main text word. Count: 3268

AUTHORS

Fabrizio Piras Ph.D.^{1*}, Federica Piras Ph.D.¹, Yoshinari Abe M.D.², Sri Mahavir Agarwal M.D.³, Ph.D., Alan Anticevic Ph.D.⁴, Stephanie Ameis M.D.^{5,6,7}, Paul Arnold M.D. Ph.D.⁸, N ria Bargall  M.D., Ph.D.^{9,10}, Marcelo C. Batistuzzo Ph.D.¹¹, Francesco Benedetti M.D.¹², Jan-Carl Beucke Ph.D.^{13,14}, Premika S.W. Boedhoe M.Sc.^{15,16}, Irene Bollettini Ph.D.¹², Silvia Brem Ph.D.¹⁷, Anna Calvo M.Sc.⁹, Kang Ik Kevin Cho Ph.D.¹⁸, Sara Dallyspezia M.D., Ph.D.¹², Erin Dickie Ph.D.¹⁹, Benjamin Adam Ely B.S.²⁰, Siyan Fan Ph.D.¹⁶, Jean-Paul Fouche M.Sc.²¹, Patricia Gruner Ph.D.⁴, Deniz A. G rsel M.Sc.²², Tobias Hauser Ph.D.¹⁶, Yoshiyuki Hirano Ph.D.²³, Marcelo Q. Hoexter M.D., Ph.D.¹¹, Mariangela Iorio Ph.D.^{1,24}, Anthony James M.D.²⁵, Janardhan Reddy M.D.³, Christian Kaufmann Ph.D.^{13,26}, Kathrin Koch Ph.D.²², Peter Kochunov Ph.D.²⁷, Jun Soo Kwon M.D., Ph.D.¹⁸, Luisa Lazaro M.D., Ph.D.^{28,29}, Christine Lochner Ph.D.²¹, Rachel Marsh Ph.D.³⁰, Akiko Nakagawa M.D., Ph.D.²³, Takashi Nakamae M.D., Ph.D.², Janardhanan C. Narayanaswamy, M.D.³, Yuki Sakai M.D., Ph.D.², Eiji Shimizu M.D., Ph.D.²³, Daniela Simon Ph.D.¹³, Helen Blair Simpson M.D., Ph.D.³⁰, Noam Soreni M.D.³¹, Philipp St mpfli Ph.D.³², Emily R. Stern Ph.D.²⁰, Philip Szeszko Ph.D.³³, Jumpei Takahashi M.D.²³, Ganesan Venkatasubramanian M.D., Ph.D.³, Zhen Wang M.D., Ph.D.³⁴, Je-Yeon Yun M.D.¹⁸ Ph.D., ENIGMA OCD Working Group*, Dan J. Stein M.D., Ph.D.²¹, Neda Jahanshad Ph.D.³⁵, Paul M. Thompson Ph.D.³⁵, Odile A. van den Heuvel M.D., PhD.^{15,16} and Gianfranco Spalletta M.D., PhD^{1,36*}

*ENIGMA OCD Working Group also includes:

Francesca Assogna Ph.D.¹, Nerisa Banaj Ph.D.¹, Rosa Calvo M.D.^{29,37}, Ph.D., Valentina Ciullo Ph.D.¹, Stella J. de Wit M.D.^{15,16}, Morgan Hough B.A.³⁸, Masaru Kuno M.D., Ph.D.²³, Euripedes C. Miguel M.D., Ph.D.¹¹, Astrid Morer M.D., Ph.D.²⁸, Christopher Pittenger M.D., Ph.D.⁴, Sara Poletti Ph.D.¹², Enrico Smeraldi M.D.¹²,

João R. Sato Ph.D.³⁹, Aki Tsuchiyagaito Ph.D.²³, Susanne Walitza Ph.D.¹⁷, Ysbrand D. van der Werf Ph.D.¹⁶, Daniela Vecchio Ph.D.¹, Mojtaba Zarei M.D. Ph.D.⁴⁰

***Correspondence to f.piras@hsantalucia.it; g.spalletta@hsantalucia.it**

Laboratory of Neuropsychiatry, Department of Clinical and Behavioral Neurology, IRCCS Santa Lucia Foundation, Rome, Italy.

Address: Via Ardeatina 306, Rome, Italy

Telephone: +390651501100

AFFILIATIONS

1. Laboratory of Neuropsychiatry, Department of Clinical and Behavioral Neurology, IRCCS Santa Lucia Foundation, Rome, Italy
2. Department of Psychiatry, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan
3. Obsessive-Compulsive Disorder (OCD) Clinic, Department of Psychiatry, National Institute of Mental Health & Neurosciences, Bangalore, India
4. Department of Psychiatry, Yale University School of Medicine, New Haven, Connecticut, U.S.A.
5. Child, Youth and Emerging Adult Program, Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health (CAMH), Toronto, ON, Canada
6. Department of Psychiatry, Hospital for Sick Children, Toronto, ON, Canada
7. Department of Psychiatry, University of Toronto, Toronto, ON, Canada
8. Mathison Centre for Mental Health Research & Education, Hotchkiss Brain Institute, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada
9. Magnetic Resonance Image Core Facility, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain
10. Centre de Diagnostic per la Imatge (CDIC), Hospital Clínic de Barcelona, Spain
11. Instituto e Departamento de Psiquiatria, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil

12. Psychiatry and Clinical Psychobiology, Division of Neuroscience, Scientific Institute Ospedale San Raffaele, Milano, Italy
13. Department of Psychology, Humboldt-Universität zu Berlin, Germany
14. K8 Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden
15. Amsterdam University Medical Centers, Vrije Universiteit, Department of Psychiatry, Amsterdam Neuroscience, Amsterdam, The Netherlands
16. Amsterdam university medical centers, Vrije Universiteit, Department of Anatomy & Neurosciences, Amsterdam Neuroscience, Amsterdam, The Netherlands
17. Department of Child and Adolescent Psychiatry and Psychotherapy, Psychiatric Hospital, University of Zurich, Zurich, Switzerland
18. Institute of Human Behavioral Medicine, SNU-MRC, Seoul, Republic of Korea
19. Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health (CAMH), Toronto, ON, Canada
20. Department of Neuroscience and Graduate School, Icahn School of Medicine at Mount Sinai, New York, NY, U.S.A.
21. SAMRC Unit on Anxiety & Stress Disorders, Department of Psychiatry and Neuroscience Institute, University of Cape Town, South Africa
22. Department of Neuroradiology, Klinikum rechts der Isar, Technische Universität München, Germany
23. Research Center for Child Mental Development, Chiba University, Chiba, Japan
24. Center of Excellence on Aging and Translational Medicine - CeSI-MeT, Chieti, Italy
25. Department of Psychiatry, Oxford University, Oxford, U.K.
26. Department of Psychology, Freie Universität zu Berlin, Germany
27. Maryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD, U.S.A.
28. Department of Child and Adolescent Psychiatry and Psychology, Institute of Neurosciences, Hospital Clínic Universitari, Barcelona, Spain
29. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain
30. Columbia University Medical College, Columbia University, New York, NY, U.S.A. & the New York State Psychiatric Institute, New York, NY, U.S.A.

31. Pediatric OCD Consultation Clinic, Anxiety Treatment and Research Center, McMaster University, Hamilton, Ontario, Canada
32. MR-Center of the Department of Psychiatry, Psychotherapy and Psychosomatics and the Department of Child and Adolescent Psychiatry, Psychiatric Hospital of the University of Zurich, Zurich, Switzerland
33. Department of Psychiatry, Icahn School of Medicine at Mount Sinai, NY, U.S.A.
34. Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, PR China
35. Imaging Genetics Center, Mark and Mary Stevens Neuroimaging & Informatics Institute, Keck School of Medicine of the University of Southern California, Marina del Rey, U.S.A.
36. Division of Neuropsychiatry, Menninger Department of Psychiatry and Behavioral Sciences, Baylor College of Medicine, Houston, TX, U.S.A.
37. CIBERSAM, Barcelona, Spain
38. Highfield Unit, Warneford Hospital, Oxford, UK
39. Center of Mathematics, Computation, and Cognition, Universidade Federal do ABC, Santo André, São Paulo, Brazil
40. Institute of Medical Science and Technology, Shahid Beheshti University, Tehran, Iran

KEY POINTS

Question: Do patients with Obsessive-Compulsive Disorder (OCD) show white matter microstructural alterations, and are these alterations related to clinical features?

Findings: Data from 19 sites of the ENIGMA-OCD Consortium were included, involving 700 adult patients and 645 adult controls, 174 pediatric patients and 144 pediatric controls. Diffusion tensor imaging data were meta-analyzed using a harmonized data processing and analysis protocol. Adult, but not pediatric,

patients showed alterations in the sagittal stratum and posterior thalamic radiation; sagittal stratum differences were associated with clinical features.

Meaning: Microstructural abnormalities found in adult but not in the pediatric cohort, are related to illness duration and medication status.

ABSTRACT

Importance: Microstructural alterations in cortico-subcortical connections are thought to be present in Obsessive-Compulsive Disorder (OCD). However, prior studies have yielded inconsistent findings, perhaps because small sample sizes provided insufficient power to detect subtle abnormalities.

Objective: To investigate microstructural white matter alterations and their relation to clinical features in the largest dataset of adult and pediatric OCD to date.

Design, Setting, and Participants: In this cross-sectional case-control magnetic resonance study, we investigated diffusion tensor imaging metrics from 700 adult patients and 645 adult controls, as well as 174 pediatric patients and 144 pediatric controls across 19 sites participating in the ENIGMA-OCD Working Group.

Main Outcomes and Measures: We extracted measures of fractional anisotropy (FA) as main outcome, and mean diffusivity, radial diffusivity, and axial diffusivity as secondary outcomes for 25 white matter regions. We meta-analyzed patient-control group differences (Cohen's d) across sites, after adjusting for age and sex, and investigated associations with clinical characteristics.

Results: Adult OCD patients showed significant FA reduction in the sagittal stratum ($d=-0.21$, $z=-3.21$, $p=0.001$) and posterior thalamic radiation ($d=-0.26$, $z=-4.57$, $p<0.0001$). In the sagittal stratum only, lower FA was associated with a younger age of onset ($z=2.71$, $p=0.006$), longer duration of illness ($z=-2.086$, $p=0.036$) and a higher percentage of medicated patients in the cohorts studied ($z=-1.98$, $p=0.047$). No significant association with

symptom severity was found. Pediatric OCD patients did not show any detectable microstructural abnormalities compared to matched controls.

Conclusions and Relevance: Microstructural alterations in projection and association fibers to posterior brain regions were found in adult OCD, and related to disease course and medication status. Such results are relevant to models positing deficits in connectivity as a crucial mechanism in OCD.

INTRODUCTION

Abnormalities in cerebral white matter (WM) are relevant to models of anomalous brain circuitry that posit deficits in connectivity in obsessive-compulsive disorder (OCD)^{1,2}. OCD has a childhood onset in over 50% of all cases, and most childhood-onset OCD cases persist into adulthood³. Diffusion tensor imaging (DTI) allows the study of WM at the microstructural level through the analysis of intrinsic, three-dimensional diffusion properties of water within brain tissues⁴. Prior DTI studies in OCD⁵⁻⁷ suggest that microstructural alterations are present in a number of WM areas. However, results across studies are inconsistent, with contrasting or conflicting effects of OCD on DTI metrics⁸. Sources of heterogeneity may include methodological factors (e.g., imaging acquisition and data processing), clinical characteristics, and variations in demographic or socioeconomic factors. More importantly, sample size variations may impact reported findings, as small studies may have insufficient power to detect subtle alterations⁹. Brain imaging consortia offer new opportunities, pooling data and findings from around the world to achieve an appropriate sample size. The OCD working group of the Enhancing Neuro-Imaging Genetics through Meta-Analysis (ENIGMA) consortium¹⁰, is one such collaboration. Previous findings from the working group focused on subcortical and cortical brain grey matter abnormalities, using subcortical volumes, cortical thickness and surface area quantification algorithms. An initial analysis of data from 3,589 individuals showed distinct subcortical volume abnormalities in adults (smaller hippocampal and larger pallidal volumes) and unmedicated children (larger thalamic volume) with OCD¹¹. The second study focused on cortical

grey matter differences and showed a lower surface area for the transverse temporal cortex and a thinner inferior parietal cortex in adult patients. In pediatric OCD patients compared to healthy controls, significantly thinner inferior and superior parietal cortices were found¹². Medication status was associated with structural differences in both pediatric and adult OCD.

Here we aimed to investigate WM microstructural alterations in adult and pediatric OCD using data from the ENIGMA OCD working group, in the subset of participants that had collected diffusion MRI. DTI metrics in 700 adult patients were compared to those of 645 adult controls, and separately, 174 pediatric patients were compared to 144 pediatric controls. Analyses also aimed to investigate associations between WM microstructure and demographic and clinical variables. As prior meta-analytic findings in frontal and callosal regions have been inconsistent (with either higher¹ or lower^{1,5} fractional anisotropy -FA- in anterior midline tracts), with more homogenous findings for fronto-temporal and fronto-parietal intra-hemispheric bundles, we expected to find microstructural alterations (as reflected by lower FA^{1,5,6,8}) in the long tracts connecting frontal regions to posterior temporal, parietal and occipital association cortices.

METHODS

Study dataset

The ENIGMA-OCD Working Group includes 19 international research institutes. Previous literature (including studies from the present Working Group^{11,12}) showed different patterns of effects in pediatric and adult cohorts; thus, we performed separate meta-analyses for adult and pediatric data. Globally, we analyzed data from 1,345 adults (including 700 OCD patients and 645 controls, aged ≥ 18) and 318 children (including 174 OCD and 144

controls). Patients were administered the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS)¹³ and the Child Yale-Brown Obsessive-Compulsive Scale (CY-BOCS)¹⁴ to assess symptom severity. These scales are clinician-rated, 10-item scales, with each item rated from 0 (no symptoms) to 4 (extreme symptoms; total range, 0 to 40), with separate subtotals for severity of obsessions and compulsions. **Tables 1** and **2** show the demographic and clinical characteristics of the participants from each site.

All local IRBs approved the use of measures extracted from completely anonymized data.

Image Acquisition and Processing

Harmonized preprocessing, including brain extraction, eddy current correction, movement correction, echo-planar imaging-induced distortion correction and tensor fitting, was carried out at each site, using protocols and quality control pipelines provided by the ENIGMA-DTI working group (<http://enigma.ini.usc.edu/protocols/dti-protocols/>) and already employed to pool harmonized DTI analyses from around the world¹⁵⁻¹⁷.

Once tensors were estimated, each site conducted a harmonized image analysis for FA quantification using the ENIGMA-DTI protocol, consisting of the tract-based spatial statistics (TBSS)¹⁸ analytic method modified to project individual FA values to the ENIGMA-DTI skeleton. Tract-wise regions of interest (ROIs), derived from the Johns Hopkins University (JHU)¹⁹ white matter parcellation atlas, were used to extract the mean FA across the full skeleton and mean FA values for 25 ROIs.

Diffusivity measures (i.e., mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD)) were also derived for secondary analysis. In the main analyses, we combined left and right regions of interest (ROI) across

hemispheres, as we had no *a priori* hypotheses regarding lateralized effects on FA.

Statistical Analysis

At each site, Cohen's *d* effect sizes were calculated for differences in FA between patients and healthy controls. Age, sex, age-by-sex interaction and quadratic covariates of age² and age²-by-sex interaction were included in the model, as linear and nonlinear age and sex interactions have been reported for FA¹⁵. Subsequently, a random effects meta-analysis was run at the coordinating site using Comprehensive Meta-Analysis (CMA, version 2, Biostat, Englewood, NJ) to combine individual site effect sizes. Heterogeneity scores (*I*²; lower values indicate lower variance in the effect size estimates across studies) were also computed for each test.

Effect sizes are reported as overall Cohen's *d* values for case/control effects and Z-scores for quantitative effects from linear regressions. To control the probability of false positives due to multiple comparisons, effects of FA differences between cases and controls were considered significant if they survived the Bonferroni correction threshold of 0.05/25 (the 25 considered tracts)= 0.002. The stability of the overall effect size estimate was tested using a 'leave one out' sensitivity analysis. This analysis shows how the overall effect size changes if one dataset at a time is removed, assessing whether potential results are site-dependent. Furthermore, to ascertain whether the estimated effect size varied as a function of clinical characteristics, mixed effects meta-regressions were performed on diffusion parameters using age, age of onset, duration of illness, illness severity and percentage of medicated patients in the patients' dataset as regressors. The

influence of medication status was also explored through a mixed-effects sub-group analysis, comparing effect sizes in medicated (n=8) and unmedicated (n=3) patient cohorts.

RESULTS

Demographics and clinical characteristics of the participants in each site are shown in Tables 1 and 2.

-----Insert Tables 1 and 2 about here-----

Adult Cohort

Table 3 indicates the five out of 25 regions with marginally lower FA in patients compared to controls. These are the *genu* of the corpus callosum, the posterior *corona radiata*, the posterior thalamic radiation (PTR), the sagittal stratum (SS) and the uncinata fasciculus (see Figure 1).

-----Insert Table 3 and Figure1 about here-----

FA in the SS ($d=-0.21$, $z=-3.21$, $p=0.001$) and the PTR ($d=-0.261$, $z=-4.57$, $p<0.0001$) survived correction for multiple comparisons. For these two regions, heterogeneity values were the lowest and did not reach statistical significance ($I^2=24.43$, $p=0.21$ and $I^2=6.61$, $p=0.38$, respectively) implying that effect size estimates across sites were highly consistent for regions where results survived correction. A sensitivity analysis for the PTR showed that the OCD effect was robust, yet, for the SS, Bonferroni-corrected significance was lost after removing single datasets. However, when more homogenous cohorts (i.e., exclusively medicated patients) were considered,

the effect of OCD diagnosis remained significant across combinations of sites.

We also analyzed diffusivity measures (i.e., MD, AD and RD) in the entire dataset. OCD patients showed higher RD in the PTR and SS, but neither result survived the Bonferroni correction threshold ($d=0.16$, $p=0.002$ for PTR and $d=0.21$, $p=0.007$ for SS). No significant results were found for MD or AD. Meta-regressions were run in regions where a significant effect of group emerged (i.e., PTR and SS). In the SS of adults diagnosed with OCD, lower FA was significantly associated with younger age of onset ($z=2.71$, $p=0.006$), longer duration of illness ($z=-2.09$, $p=0.036$) and a higher percentage of medicated patients ($z=-1.98$, $p=0.047$; see Figure 2). Mixed effects subgroups analysis showed a significant difference ($Q\text{-value}_{(df=1)}=5.27$, $p=0.022$) between the effect sizes in medicated ($N=544$, $d=-0.274$, $p<.0001$) and unmedicated ($N=158$, $d=0.046$, $p=0.72$) patients. No relationship was found between FA values of the PTR and clinical measures. No relationship was found between FA and YBOCS scores.

-----Insert Figure 2 about here-----

Pediatric Cohort

In the pediatric cohort, patients showed no detectable FA abnormalities in any of the regions studied (see Table 4 for statistical details).

DISCUSSION

In the largest coordinated meta-analysis of WM in OCD to date, we demonstrated specific regional WM alterations, in adults with OCD with lower FA in the posterior thalamic radiation (PTR) and sagittal stratum (SS). Meta-regression indicated that lower FA in the SS is associated with younger age at onset, longer duration of illness, and being on medication, but not with symptom severity - implying that, as with cortical thickness and subcortical volumes in OCD, the observed alterations may be markers of the disorder. Findings were significant across the combinations of datasets in the sensitivity analysis for the PTR, while for the SS the effect remained significant when solely medicated patients were considered. We did not find case-control differences in WM microstructure of pediatric subjects.

A role for cerebral WM and oligodendrocytes (the myelinating cells of the central nervous system) in the pathophysiology of many psychiatric disorders has been supported by growing research evidence^{6,20}, suggesting abnormalities of myelination status as a possible pathogenic mechanism²¹. Specifically, altered myelin-related maturational growth may explain the enhanced risk for psychiatric disorders during the transition from childhood to adulthood^{22,23}, an age window of intense ongoing brain development²¹.

Although FA is a general measure of microstructure - including variation in regional myelination levels, such as axon demyelination or loss, myelin loss or increased extracellular space - it does not provide a physiologically specific explanation of WM abnormalities²⁴. In our study, higher RD in the same bundles, although at a trend level, supports the hypothesis that lower FA reflects a disruption of myelin sheaths²⁵, given that RD is a putative myelin marker²⁴. The association between myelin degradation in the SS and

longer illness duration together with the absence of a detectable alteration in pediatric patients, suggests that neuroplastic changes are epiphenomena of prolonged symptomatology, since compulsively engaging in a particular behavior or cognitive process has been suggested to alter brain structure^{26,27}. Moreover, symptoms indicative of obsessive–compulsive traits are related to individual myelination over time even in otherwise healthy samples. This suggests that the mechanisms underlying compulsivity have long-lasting effects on brain development, possibly affecting myelination trajectories during adolescence, with lasting effects into adulthood²¹. Alternatively, the paucity of extrinsic factors regulating the development of myelinating glia²⁸ could have driven the altered myelination and its association with prolonged illness. Indeed, impoverished environment is both the cause and the consequence of mental illness in general, and of compulsivity in particular²⁹. Drug-induced reductions in the FA of several WM tracts may be part of the mechanism of action of drug treatments in OCD³⁰. Long-term treatment exposure may negatively influence the proliferation of oligodendrocytes and their myelination of axons^{25,31–33}. Drug treatments for psychiatric disorders may act in part through effects on myelinating glia, as oligodendrocytes have neurotransmitter receptors for glutamate, serotonin, and dopamine. Drugs acting through these neurotransmitter systems can exert actions on myelination that may be detrimental^{30,33} or beneficial³⁴. In the present study, medication effects could not be explored in great detail, but lower FA in the SS was related to medication status and observed only in the medicated patient cohort. Moreover, the effect remained significant across combinations of datasets when only medicated patients were considered, strengthening the hypothesis that medication impacts WM microstructure.

Although the mechanisms of altered WM microstructure in OCD are far from clear, a number of lines of research^{35,36} and prior genetic association studies have described specific genes related to myelination³⁷, while there is evidence that genetic variants related to glutamatergic, dopaminergic, and neurodevelopmental pathways determine WM microstructure in children and adolescents with OCD³⁸. Although no genes related to myelination have yet reached genome wide-significance, given preliminary findings from small samples, it is possible that such significance will be reached with better powered GWASs. Particularly, variants in the transcription factor of the oligodendrocyte lineage (OLIG2), involved in myelination and neurogenesis and essential in regulating the development of myelin producing cells, and in the myelin oligodendrocyte glycoprotein (MOG), implicated in maintaining myelin-axon integrity in the normal brain, have been associated with OCD in familial and association studies³⁹⁻⁴¹. Although not at a genome-wide level of evidence, a single nucleotide polymorphism in the OLIG2 gene (related to psychiatric disorders including OCD⁴⁰) was associated with reduced FA in WM regions of healthy subjects⁴², suggesting that it may confer increased risk for psychiatric illnesses through an effect on neuroanatomical connectivity. The additional observation that polymorphisms of genes codifying for myelin-promoting factors like the brain-derived neurotrophic factor (BDNF) are associated with OCD (specifically with early onset)⁴³ and particularly with WM microstructure³⁸, further suggests a role for altered myelination in the pathophysiology of the disorder. However, such interpretative framework of genetic influence on altered myelination in OCD is rather speculative and should be considered with caution, given that the cited studies are underpowered.

Axon-derived signals, like glutamate release, may also play a role in the epigenetic regulation of the transcriptional apparatus required for myelination by oligodendrocytes⁴⁴, and in the process of remyelination after damage. Glutamate-related genes are promising candidates for OCD⁴⁵⁻⁴⁷, and neuroimaging-gene association studies in animal models reinforce the hypothesis of glutamate involvement in the pathophysiology of the disorder⁴⁸. Since abnormalities in glutamate neurotransmission and homeostasis have shown to be crucial for OCD onset⁴⁹, glutamate excitotoxicity-induced damage of the myelin sheaths may further explain the decline in myelin integrity suggested here. The PTR and, to a lesser extent, the SS convey fibers principally from the thalamus, a region found to be consistently enlarged in unmedicated pediatric OCD samples¹¹ and functionally hyperactive⁵⁰ in OCD adults. This pathological hyperactivity could increase glutamate release⁵¹, and excess glutamate from thalamic projections may determine the microstructural alteration in WM bundles reported here. Several lines of evidence from magnetic resonance spectroscopy (MRS) and from clinical trials support the existence of glutamatergic hyperactivity associated with OCD⁵¹. This indicates that the disruption of microstructure in the PTR and SS may partially be a consequence of other processes (e.g., glutamate abnormalities, a potential epiphenomenon of neuronal hyperactivity in OCD circuitry) in the pathophysiology of OCD⁵¹.

Since both the PTR and the SS convey projection fibers to the posterior part of the brain, our results support the idea that OCD involves abnormalities affecting a network of regions that is more extensive than commonly believed^{1,52}. Both bundles project to posterior parietal, temporal and occipital cortices, and include many major association fibers (i.e., the inferior

longitudinal fasciculus and the inferior fronto-occipital fasciculus). Their altered microstructure may be related to the cognitive dysfunctions subtended by intra-hemispheric disconnection^{2,53}.

Notably, in our study WM microstructural alterations in OCD were associated with age at scanning. Specifically, WM alterations were observed in the adult cohort only, and were associated with longer illness duration. These findings, which were unrelated to OCD symptom severity, complement previous evidence of differences between adult and pediatric OCD patients in brain morphological^{5,12,54} and clinical⁵⁵ correlates.

There is evidence that the human brain's protracted myelination^{56,57} underpins myelin vulnerability along a continuum from early to late stages of development and disease⁵⁸. Thus, it has been suggested that pediatric OCD could be a neurodevelopmental disorder with potentially differing patterns of myelination occurring throughout life⁵⁹. Indeed, evidence in healthy subjects indicates that the psychiatric trait of compulsivity is linked to reduced myelin growth that emerges only during adolescence (being present only to a minor extent in childhood) as a result of aberrant developmental processes²¹. Intriguingly, MOG influence on myelin maturation and remodeling is different depending on the age of onset⁶⁰ in those psychiatric disorders with microstructural white matter abnormalities overlapping with OCD⁶. Thus, brain changes associated with OCD genetic vulnerability may be dynamic over time, and dependent on timing of gene expression⁶¹, neurodevelopmental stage at illness onset, and disease course afterwards. Alternatively, pediatric OCD might be a developmentally moderated expression of etiologic processes that are shared with the adult clinical phenotype.

A number of limitations of the data analyzed here deserve emphasis. First, although TBSS is a widely used method for voxel-based analysis of WM, addressing issues associated with smoothing and misalignment in DTI group analysis⁵², the technique has some limitations. Indeed, by reducing WM tracts into a skeleton, delineating the center of the tracts and projecting onto it only the highest FA value along the projection, some information might be lost⁶² and potential artifacts, resulting from misregistration, might be produced⁶³. Nevertheless, several test–retest and reliability analyses were conducted by the ENIGMA-DTI working group to ensure reproducibility of measures and effects using this TBSS approach⁶⁴. Moreover, a word of caution is needed regarding the interpretation of the neurobiological basis of DTI measures since although FA reflects the myelination, orientational coherence, and microtubular axonal structure of fibers, other *in vivo* markers not explored in the present study have been shown to be a more direct reflection of myelination status^{65,66}. Another potential limitation of the present study may lie in the differences in clinical characteristics between the studied patients, particularly in the average age of onset (which ranged from 4 to 49). Since the latter is often calculated retrospectively, a reliable and unanimous method for establishing this important effect moderator is warranted. Also, we were not able to calculate specific dosages of different medication types and analyze medication effects in terms of drug dosages or total time of treatment. Thus, our interpretation that a disruption of myelin sheaths in the PTR and SS may be a downstream consequence of prolonged medication or glutamatergic abnormalities in OCD should be viewed with caution, as the potential detrimental/normalizing effects of different medications could not be fully tested. Lastly, it is worth mentioning that while the adult cohort analysis

had sufficient power to detect the observed effect size, as the sample size was adequate to detect microstructural differences as small as $d=0.15$, the null result in the pediatric cohort may be a consequence of the relatively small sample size since the power for potentially detecting even very small differences was low (0.32). Nevertheless, this is the largest pediatric dataset investigated in a DTI study of OCD.

In summary, our results clearly indicate a key role in adult OCD of microstructural alterations in projection and association fibers to posterior brain regions. They also demonstrate the moderating effect of illness chronicity and medication on WM microstructure. Such alterations might be a downstream consequence of other processes that are more primary to the pathophysiology of the disorder and potentially related to OCD genetic vulnerability. Our meta-regression results related to duration suggest that microstructural alterations may persist along the course of the illness, although longitudinal data will be needed to confirm such trajectories. Thus, from a clinical perspective, early symptom reduction and subsequent improved functioning might counteract genetic vulnerability and promote the epigenetic regulation of myelin remodeling^{67,68} by enhancing extrinsic factors mediating myelin plasticity²⁸. However, pharmacological amelioration of symptoms should be cautiously considered, given the detrimental medication effect we observed on WM microstructure. Other therapeutic approaches, such as cognitive-behavioural⁶⁹ therapies should be strongly encouraged.

Future studies to investigate the co-occurrence of abnormal WM microstructure, GM volume and metabolic differences in OCD will shed light on the interactions and trajectories of structural and functional alterations in this condition. In particular, longitudinal designs, and collecting information

from patients at their illness onset, combined with multimodal MRI approaches such as volumetric, DTI, fMRI, and MRS will help provide an understanding of the timing and course of brain changes in OCD and provide greater insight into the mechanisms involved in various stages of OCD, including the long-term effects of medication.

REFERENCES

1. Piras F, Piras F, Caltagirone C, Spalletta G. Brain circuitries of obsessive compulsive disorder: A systematic review and meta-analysis of diffusion tensor imaging studies. *Neurosci Biobehav Rev.* 2013;37(10):2856-2877. doi:10.1016/j.neubiorev.2013.10.008
2. Calzà J, Gürsel DA, Schmitz-Koep B, et al. Altered Cortico-Striatal Functional Connectivity During Resting State in Obsessive-Compulsive Disorder. *Front psychiatry.* 2019;10:319. doi:10.3389/fpsy.2019.00319
3. Micali N, Heyman I, Perez M, et al. Long-term outcomes of obsessive-compulsive disorder: Follow-up of 142 children and adolescents. *Br J Psychiatry.* 2010;197(2):128-134. doi:10.1192/bjp.bp.109.075317
4. Basser PJ, Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. *J Magn Reson - Ser B.* 1996;111(3):209-219. doi:10.1006/jmrb.1996.0086
5. Radua J, Grau M, Van Den Heuvel OA, et al. Multimodal voxel-based meta-analysis of white matter abnormalities in obsessive-compulsive disorder. *Neuropsychopharmacology.* 2014;39(7):1547-1557. doi:10.1038/npp.2014.5
6. Jenkins LM, Barba A, Campbell M, et al. Shared white matter alterations across emotional disorders: A voxel-based meta-analysis of fractional anisotropy. *NeuroImage Clin.* 2016;12:1022-1034. doi:10.1016/j.nicl.2016.09.001
7. Koch K, Reef TJ, Rus OG, Zimmer C, Zaudig M. Diffusion tensor imaging (DTI) studies in patients with obsessive-compulsive disorder (OCD): A review. *J Psychiatr Res.* 2014;54(1):26-35. doi:10.1016/j.jpsychires.2014.03.006

8. Eng GK, Sim K, Chen SHA. Meta-analytic investigations of structural grey matter, executive domain-related functional activations, and white matter diffusivity in obsessive compulsive disorder: An integrative review. *Neurosci Biobehav Rev.* 2015;52:233-257.
doi:10.1016/j.neubiorev.2015.03.002
9. Melicher T, Horacek J, Hlinka J, et al. White matter changes in first episode psychosis and their relation to the size of sample studied: A DTI study. *Schizophr Res.* 2015;162(1-3):22-28. doi:10.1016/j.schres.2015.01.029
10. Thompson PM, Dennis EL, Gutman BA, et al. ENIGMA and the individual: Predicting factors that affect the brain in 35 countries worldwide. *Neuroimage.* 2017;145:389-408. doi:10.1016/j.neuroimage.2015.11.057
11. Boedhoe PSW, Schmaal L, Abe Y, et al. Distinct subcortical volume alterations in pediatric and adult OCD: A worldwide meta- and mega-analysis. *Am J Psychiatry.* 2017;174(1):60-70.
doi:10.1176/appi.ajp.2016.16020201
12. Boedhoe PSW, Schmaal L, Abe Y, et al. Cortical abnormalities associated with pediatric and adult obsessive-compulsive disorder: Findings from the enigma obsessive-compulsive disorder working group. *Am J Psychiatry.* 2018;175(5):453-462. doi:10.1176/appi.ajp.2017.17050485
13. Goodman WK, Price LH, Rasmussen S a, et al. The Yale-Brown Obsessive Compulsive Scale. I. Development, use, and reliability. *Arch Gen Psychiatry.* 1989;46(11):1006-1011.
doi:10.1001/archpsyc.1989.01810110048007
14. Scahill L, Riddle MA, McSwiggin-Hardin M, et al. Children's Yale-Brown Obsessive Compulsive Scale: Reliability and validity. *J Am Acad Child Adolesc Psychiatry.* 1997;36(6):844-852. doi:10.1097/00004583-

199706000-00023

15. Kelly S, Jahanshad N, Zalesky A, et al. Widespread white matter microstructural differences in schizophrenia across 4322 individuals: Results from the ENIGMA Schizophrenia DTI Working Group. *Mol Psychiatry*. 2018;23(5). doi:10.1038/mp.2017.170
16. van Velzen LS, Kelly S, Isaev D, et al. White matter disturbances in major depressive disorder: a coordinated analysis across 20 international cohorts in the ENIGMA MDD working group. *Mol Psychiatry*. 2019;in press. doi:10.1038/s41380-019-0477-2
17. Villalón-Reina JE, Martínez K, Qu X, et al. Altered white matter microstructure in 22q11.2 deletion syndrome: a multisite diffusion tensor imaging study. *Mol Psychiatry*. 2019;in press. doi:10.1038/s41380-019-0450-0
18. Smith SM, Jenkinson M, Johansen-Berg H, et al. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *Neuroimage*. 2006;31(4):1487-1505. doi:10.1016/j.neuroimage.2006.02.024
19. Mori S, Oishi K, Jiang H, et al. Stereotaxic white matter atlas based on diffusion tensor imaging in an ICBM template. *Neuroimage*. 2008;40(2):570-582. doi:10.1016/j.neuroimage.2007.12.035
20. Nave K-A, Ehrenreich H. Myelination and Oligodendrocyte Functions in Psychiatric Diseases. *JAMA Psychiatry*. 2014;71(5):582-584. doi:10.1001/jamapsychiatry.2014.189
21. Ziegler G, Hauser TU, Moutoussis M, et al. Compulsivity and impulsivity traits linked to attenuated developmental frontostriatal myelination trajectories. *Nat Neurosci*. 2019;22:992-999. doi:10.1038/s41593-019-0394-3

22. Kessler RC, Angermeyer M, Anthony JC, et al. Lifetime prevalence and age-of-onset distributions of mental disorders in the World Health Organization's World Mental Health Survey Initiative. *World Psychiatry*. 2007;6(3):168-176.
23. Paus T, Keshavan M, Giedd JN. Why do many psychiatric disorders emerge during adolescence? *Nat Rev Neurosci*. 2008;9(12):947-957. doi:10.1038/nrn2513
24. Song SK, Sun SW, Ju WK, Lin SJ, Cross AH, Neufeld AH. Diffusion tensor imaging detects and differentiates axon and myelin degeneration in mouse optic nerve after retinal ischemia. *Neuroimage*. 2003;20(3):1714-1722. doi:10.1016/j.neuroimage.2003.07.005
25. Rosso IM, Olson EA, Britton JC, et al. Brain white matter integrity and association with age at onset in pediatric obsessive-compulsive disorder. *Biol Mood Anxiety Disord*. 2014;4(1):13. doi:10.1186/s13587-014-0013-6
26. Maia T V, Cooney RE, Peterson BS. The neural bases of obsessive - Compulsive disorder in children and adults. *Dev Psychopathol*. 2008;20(4):1251-1283. doi:10.1017/S0954579408000606
27. Fields RD. White matter in learning, cognition and psychiatric disorders. *Trends Neurosci*. 2008;31(7):361-370. doi:10.1016/j.tins.2008.04.001
28. Fields RD. A new mechanism of nervous system plasticity: activity-dependent myelination. *Nat Rev Neurosci*. 2015. doi:10.1038/nrn4023
29. Kim SJ, Lewis M, Veenstra-VanderWeele J. The Developmental Neurobiology of Repetitive Behavior. In: Rubenstein JL, Rakic P, eds. *Neural Circuit Development and Function in the Healthy and Diseased Brain*. Cambridge, Massachusetts: Academic Press; 2013. doi:10.1016/B978-0-12-397267-5.00039-X

30. Benedetti F, Giacosa C, Radaelli D, et al. Widespread changes of white matter microstructure in obsessive-compulsive disorder: Effect of drug status. *Eur Neuropsychopharmacol*. 2013;23(7):581-593.
doi:10.1016/j.euroneuro.2012.07.002
31. Haroutunian V, Katsel P, Roussos P, Davis KL, Altshuler LL, Bartzokis G. Myelination, oligodendrocytes, and serious mental illness. *Glia*. 2014;62(11):1856-1877. doi:10.1002/glia.22716
32. Káradóttir R, Attwell D. Neurotransmitter receptors in the life and death of oligodendrocytes. *Neuroscience*. 2007;145(4):1426-1438.
doi:10.1016/j.neuroscience.2006.08.070
33. Bollettini I, Mazza MG, Muzzarelli L, et al. White matter alterations associate with onset symptom dimension in obsessive–compulsive disorder. *Psychiatry Clin Neurosci*. 2018;72(1):13-27.
doi:10.1111/pcn.12563
34. Fan Q, Yan X, Wang J, et al. Abnormalities of white matter microstructure in unmedicated obsessive-compulsive disorder and changes after medication. *PLoS One*. 2012;7(4):e35889.
doi:10.1371/journal.pone.0035889
35. Rosenberg DR, MacMillan SN, Moore GJ. Brain anatomy and chemistry may predict treatment response in paediatric obsessive-compulsive disorder. *Int J Neuropsychopharmacol*. 2001;4(4):179-190.
doi:10.1017/S1461145701002401
36. Smith EA, Russell A, Lorch E, et al. Increased medial thalamic choline found in pediatric patients with obsessive-compulsive disorder versus major depression or healthy control subjects: A magnetic resonance spectroscopy study. *Biol Psychiatry*. 2003;54(12):1399-1405.

doi:10.1016/S0006-3223(03)00474-8

37. Sampaio AS, Lins RMP, Daltro-Oliveira R, et al. Estudos de associação genética no transtorno obsessivo-compulsivo. *Arch Clin Psychiatry (São Paulo)*. 2013;40(5):177-190. doi:10.1590/S0101-60832013000500003
38. Gassó P, Ortiz AE, Mas S, et al. Association between genetic variants related to glutamatergic, dopaminergic and neurodevelopment pathways and white matter microstructure in child and adolescent patients with obsessive-compulsive disorder. *J Affect Disord*. 2015;186:284-292. doi:10.1016/j.jad.2015.07.035
39. Atmaca M, Onalan E, Yildirim H, Yuce H, Koc M, Korkmaz S. The association of myelin oligodendrocyte glycoprotein gene and white matter volume in obsessive-compulsive disorder. *J Affect Disord*. 2010;124(3):309-313. doi:10.1016/j.jad.2010.03.027
40. Stewart SE, Platko J, Fagerness J, et al. A genetic family-based association study of OLIG2 in obsessive-compulsive disorder. *Arch Gen Psychiatry*. 2007;64:209-214. doi:10.1016/S0084-3970(08)70879-X
41. Zai G, Bezchlibnyk YB, Richter MA, et al. Myelin oligodendrocyte glycoprotein (MOG) gene is associated with obsessive-compulsive disorder. *Am J Med Genet*. 2004;129B(1):64-68. doi:10.1002/ajmg.b.30077
42. Prata DP, Kanaan RA, Barker GJ, et al. Risk variant of oligodendrocyte lineage transcription factor 2 is associated with reduced white matter integrity. *Hum Brain Mapp*. 2013;34(9):2025-2031. doi:10.1002/hbm.22045
43. Hemmings SMJ, Kinnear CJ, Van Der Merwe L, et al. Investigating the role of the brain-derived neurotrophic factor (BDNF) val66met variant in obsessive-compulsive disorder (OCD). *World J Biol Psychiatry*. 2008;9(2):126-134. doi:10.1080/15622970701245003

44. Zonouzi M, Scafidi J, Li P, et al. GABAergic regulation of cerebellar NG2 cell development is altered in perinatal white matter injury. *Nat Neurosci*. 2015;18(5):674-682. doi:10.1038/nn.3990
45. Purty A, Nestadt G, Samuels J, Viswanath B. Genetics of obsessive-compulsive disorder. *Indian J Psychiatry*. 2019;61(1):s37-s42. doi:10.4103/psychiatry.IndianJPsychiatry_518_18
46. Stewart SE, Yu D, Scharf JM, et al. Genome-wide association study of obsessive-compulsive disorder. *Mol Psychiatry*. 2013;18(7):788-798. doi:10.1038/mp.2012.85
47. Mattheisen M, Samuels JF, Wang Y, et al. Genome-wide association study in obsessive-compulsive disorder: Results from the OCGAS. *Mol Psychiatry*. 2015;20(3):337-344. doi:10.1038/mp.2014.43
48. Grünblatt E, Hauser TU, Walitza S. Imaging genetics in obsessive-compulsive disorder: Linking genetic variations to alterations in neuroimaging. *Prog Neurobiol*. 2014;121:114-124. doi:10.1016/j.pneurobio.2014.07.003
49. Russell A, Cortese B, Lorch E, et al. Localized Functional Neurochemical Marker Abnormalities in Dorsolateral Prefrontal Cortex in Pediatric Obsessive-Compulsive Disorder. *J Child Adolesc Psychopharmacol*. 2003;13(supplement 1):31-38. doi:10.1089/104454603322126322
50. Maia TV, Cooney RE, Peterson BS. The neural bases of obsessive-compulsive disorder in children and adults. *Dev Psychopathol*. 2008. doi:10.1017/S0954579408000606
51. Pittenger C, Bloch MH, Williams K. Glutamate abnormalities in obsessive compulsive disorder: Neurobiology, pathophysiology, and treatment. *Pharmacol Ther*. 2011;132(3):314-332.

- doi:10.1016/j.pharmthera.2011.09.006
52. Gan J, Zhong M, Fan J, et al. Abnormal white matter structural connectivity in adults with obsessive-compulsive disorder. *Transl Psychiatry*. 2017;7(3). doi:10.1038/tp.2017.22
53. Garibotto V, Scifo P, Gorini A, et al. Disorganization of anatomical connectivity in obsessive compulsive disorder: A multi-parameter diffusion tensor imaging study in a subpopulation of patients. *Neurobiol Dis*. 2010;37(2):468-476. doi:10.1016/j.nbd.2009.11.003
54. Pujol J, Soriano-Mas C, Alonso P, et al. Mapping structural brain alterations in obsessive-compulsive disorder. *Arch Gen Psychiatry*. 2004;61(7):720-730. doi:10.1001/archpsyc.61.7.720
55. Geller DA, Biederman J, Faraone S, et al. Developmental aspects of obsessive compulsive disorder: Findings in children, adolescents, and adults. *J Nerv Ment Dis*. 2001;189(7):471-477. doi:10.1097/00005053-200107000-00009
56. de Graaf-Peters VB, Hadders-Algra M. Ontogeny of the human central nervous system: What is happening when? *Early Hum Dev*. 2006;82(4):257-266. doi:10.1016/j.earlhumdev.2005.10.013
57. Bartzokis G. b. Brain myelination in prevalent neuropsychiatric developmental disorders: Primary and comorbid addiction. *Adolesc Psychiatry (Hilversum)*. 2005;29:55-96.
58. Sherin JE, Bartzokis G. Human brain myelination trajectories across the life span: Implications for CNS function and dysfunction. In: Masoro EJ, Austad SN, eds. *Handbook of the Biology of Aging*. Cambridge, MA, US: Academic Press; 2011:333-346. doi:10.1016/B978-0-12-378638-8.00015-4
59. Gruner P, Vo A, Ikuta T, et al. White matter abnormalities in pediatric

- obsessive-compulsive disorder. *Neuropsychopharmacology*. 2012;37(12):2730-2739. doi:10.1038/npp.2012.138
60. Wu F, Kong L, Zhu Y, et al. The influence of myelin oligodendrocyte glycoprotein on white matter abnormalities in different onset age of drug-naïve depression. *Front Psychiatry*. 2018;9(186). doi:10.3389/fpsy.2018.00186
61. Douet V, Chang L, Cloak C, Ernst T. Genetic influences on brain developmental trajectories on neuroimaging studies: From infancy to young adulthood. *Brain Imaging Behav*. 2014;8(2):234-250. doi:10.1007/s11682-013-9260-1
62. Zalesky A. Moderating registration misalignment in voxelwise comparisons of DTI data: A performance evaluation of skeleton projection. *Magn Reson Imaging*. 2011;29(1):111-125. doi:10.1016/j.mri.2010.06.027
63. Schwarz CG, Reid RI, Gunter JL, et al. Improved DTI registration allows voxel-based analysis that outperforms Tract-Based Spatial Statistics. *Neuroimage*. 2014;94:65-78. doi:10.1016/j.neuroimage.2014.03.026
64. Acheson A, Wijtenburg SA, Rowland LM, et al. Reproducibility of tract-based white matter microstructural measures using the ENIGMA-DTI protocol. *Brain Behav*. 2017;7(2):e00615. doi:10.1002/brb3.615
65. Schmierer K, Scaravilli F, Altmann DR, Barker GJ, Miller DH. Magnetization transfer ratio and myelin in postmortem multiple sclerosis brain. *Ann Neurol*. 2004;56(3):407-415. doi:10.1002/ana.20202
66. Turati L, Moscatelli M, Mastropietro A, et al. In vivo quantitative magnetization transfer imaging correlates with histology during de- and remyelination in cuprizone-treated mice. *NMR Biomed*. 2015;28(3):327-337. doi:10.1002/nbm.3253

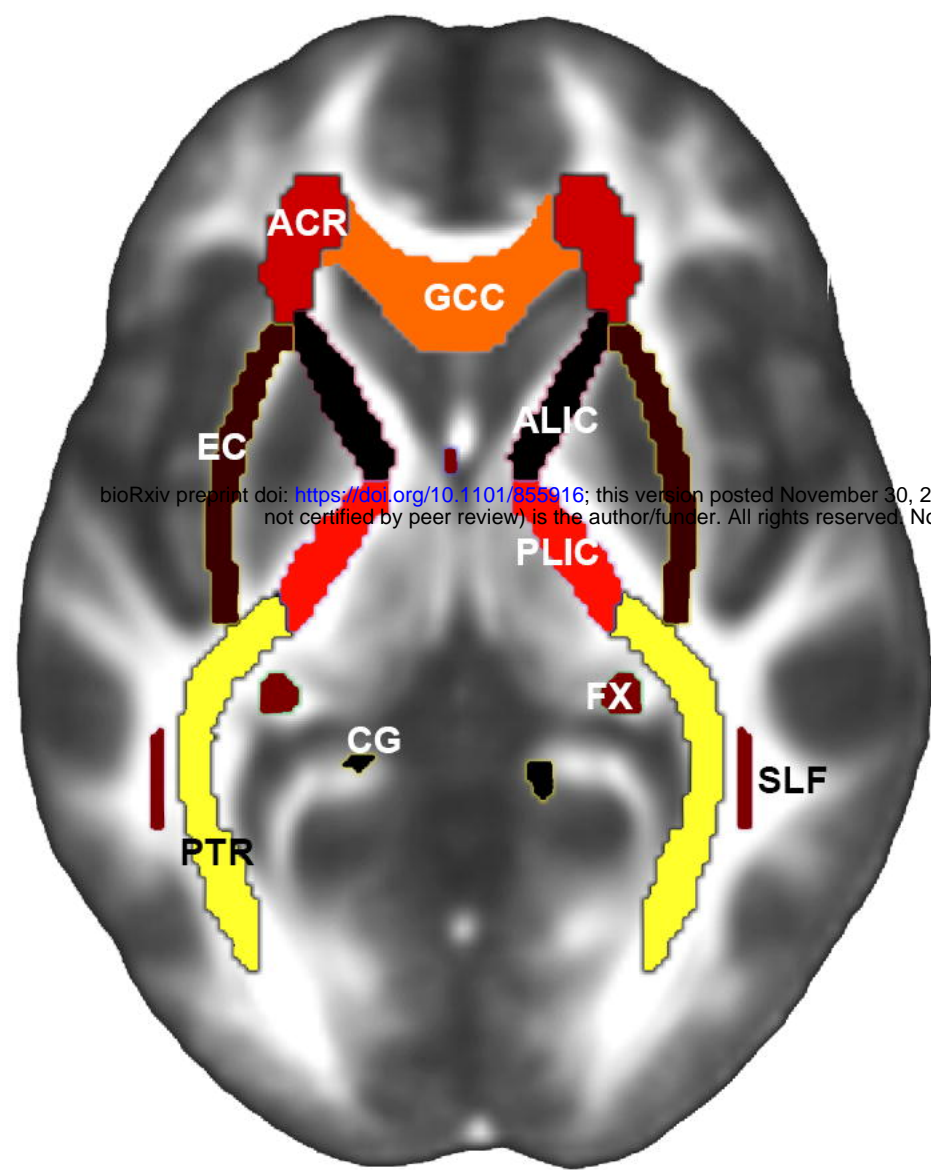
67. Brouwer RM, Panizzon MS, Glahn DC, et al. Genetic influences on individual differences in longitudinal changes in global and subcortical brain volumes: Results of the ENIGMA plasticity working group. *Hum Brain Mapp.* 2017;38(9):4444-4458. doi:10.1002/hbm.23672
68. Lee PR. Regulation of myelin genes implicated in psychiatric disorders by functional activity in axons. *Front Neuroanat.* 2009;3(4):1-8. doi:10.3389/neuro.05.004.2009
69. Fullana MA, Zhu X, Alonso P, et al. Basolateral amygdala-ventromedial prefrontal cortex connectivity predicts cognitive behavioural therapy outcome in adults with obsessive-compulsive disorder. *J Psychiatry Neurosci.* 2017;42(6):378-385. doi:10.1503/jpn.160215

FIGURE CAPTION

Figure 1: Left Panel - Fractional anisotropy (FA) differences between OCD patients and healthy controls for 25 white matter (WM) regions. Gradient bar indicates Cohen's *d* effect sizes after meta-analysis. * Threshold for significance after correction for multiple comparisons using Bonferroni correction ($P.05/25 = 0.002$). Right Panel- Cohen's *d* effect sizes after meta-analysis, after including age, sex, age \times sex, age² and age² \times sex as covariates. Error bars represent 95% confidence intervals. Significant regions after adjusting for multiple regions tested are highlighted in orange.

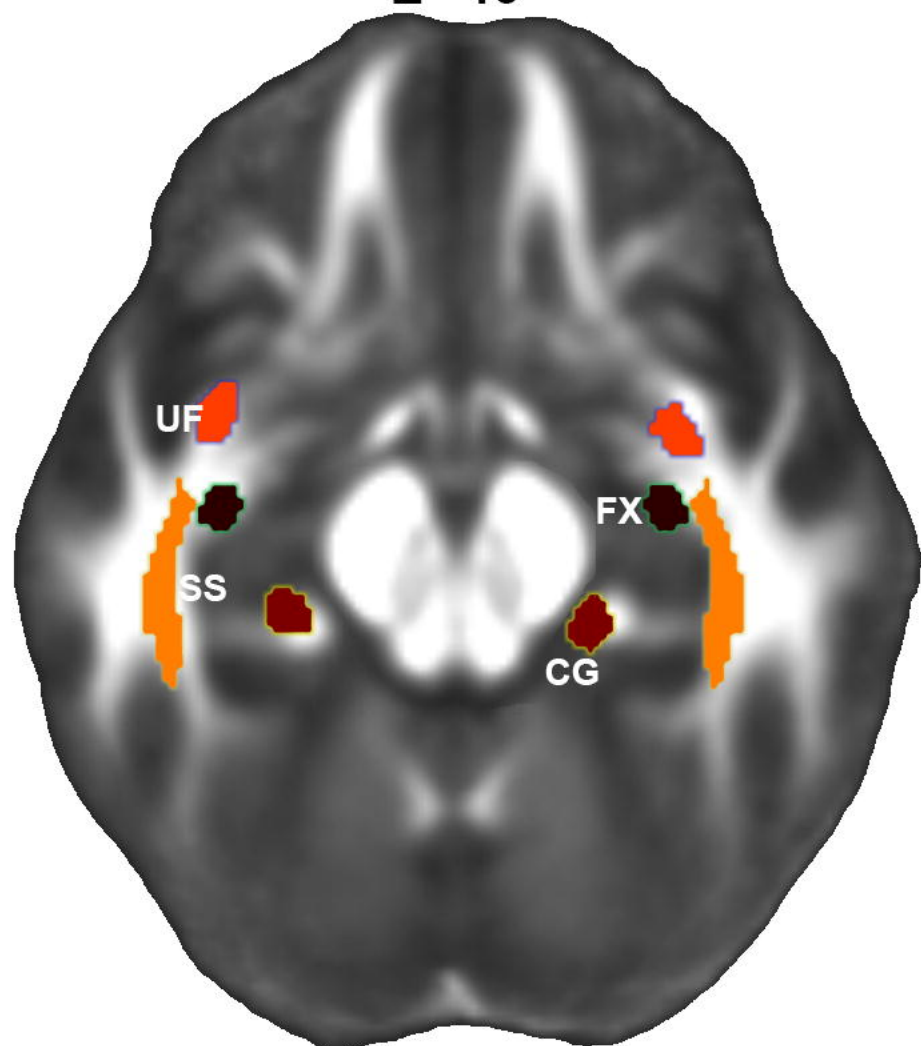
Figure 2: Association between FA reduction (in OCD adult patients) in the sagittal stratum and illness duration (*green dots*), age of onset (*blue dots*) and percentage of medicated patients across the 11 ENIGMA-OCD sites. Sphere magnitude indicates sample size.

Z=1

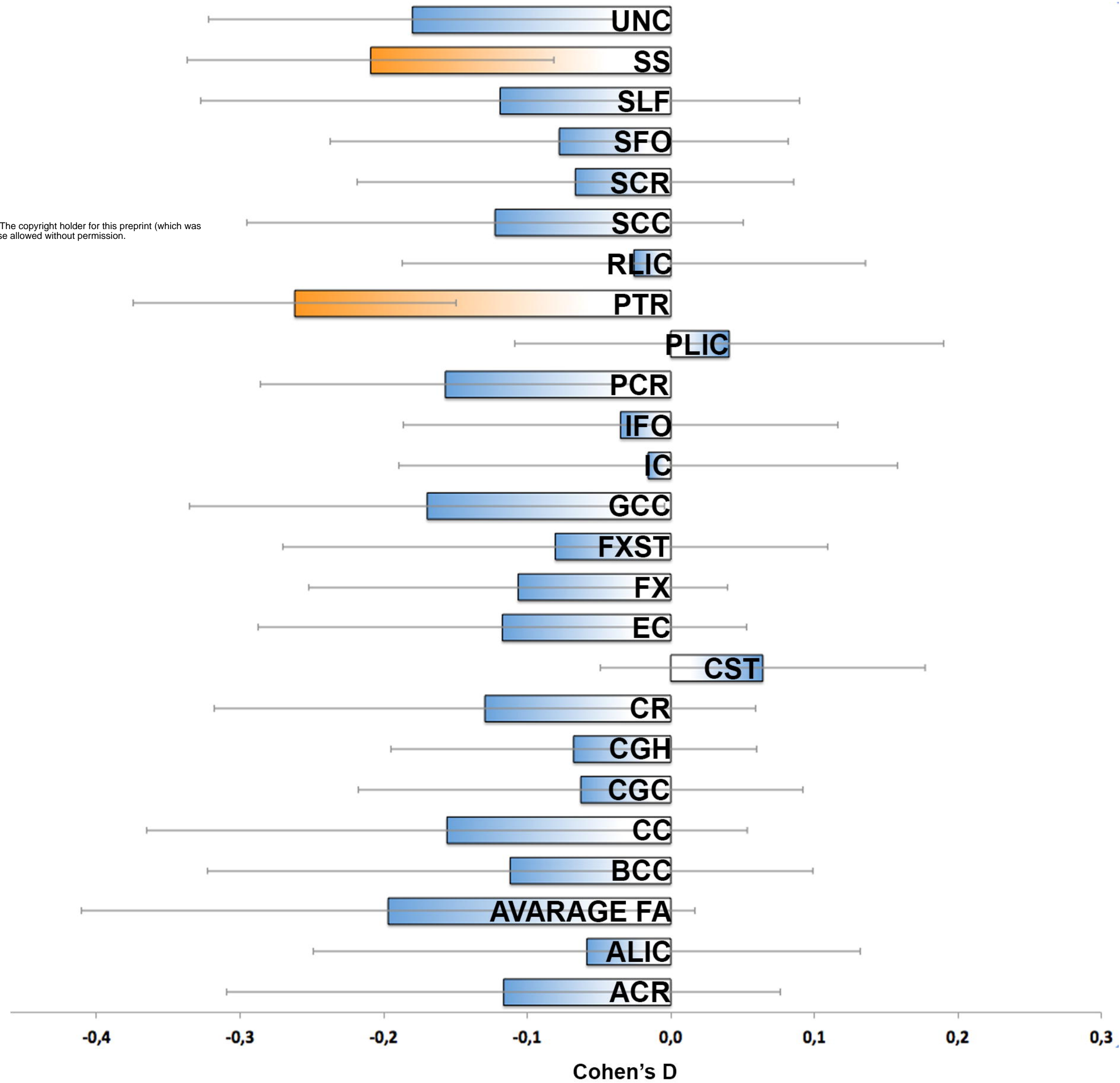
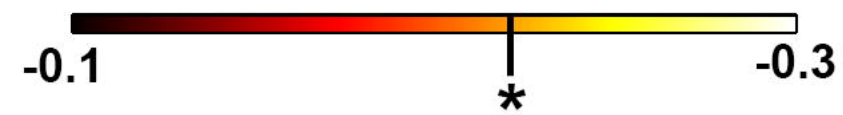


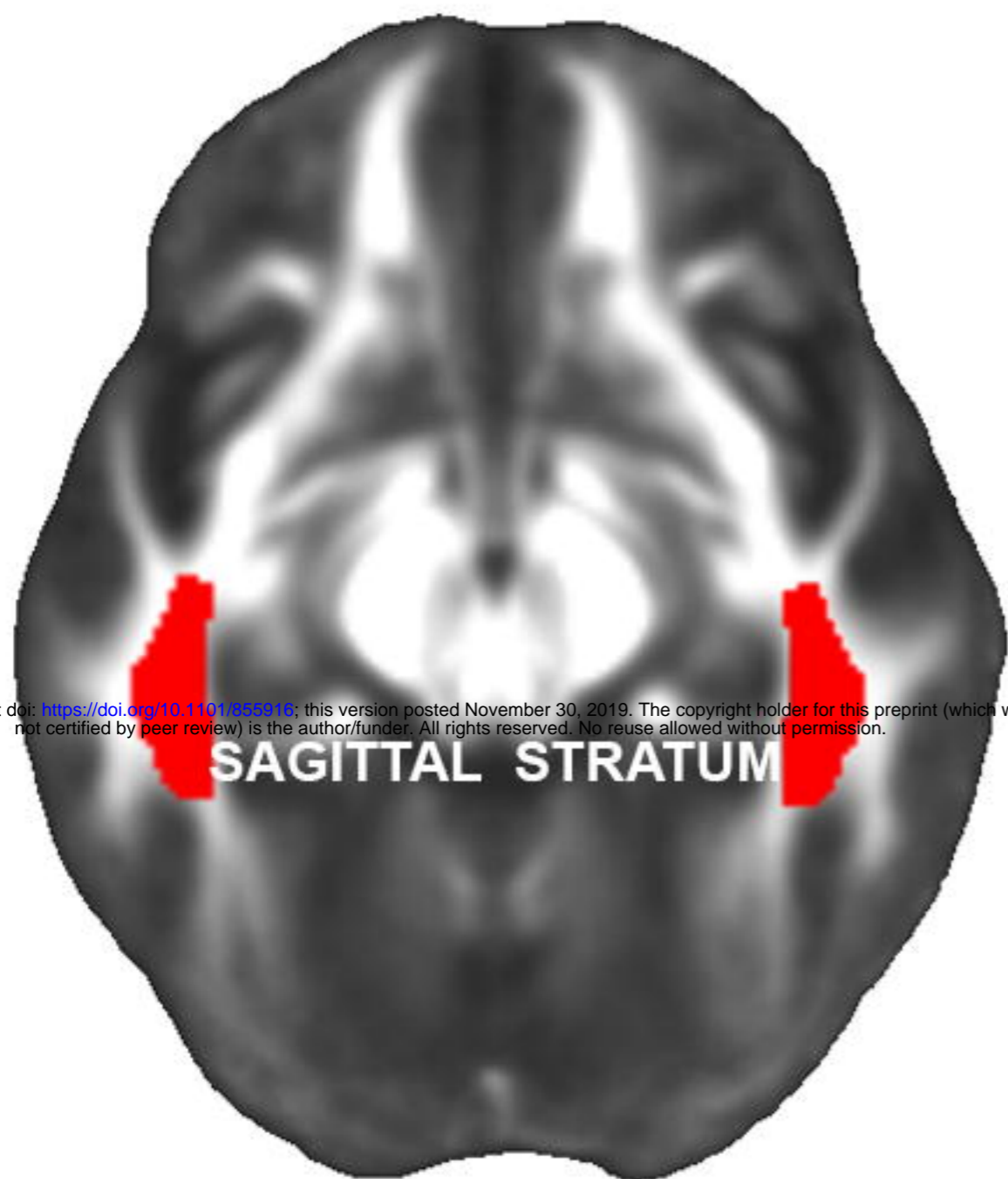
bioRxiv preprint doi: <https://doi.org/10.1101/050916>; this version posted November 30, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Z=-15



Cohen's D





bioRxiv preprint doi: <https://doi.org/10.1101/255916>; this version posted November 30, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

