The American Journal of Psychiatry

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Journal:	*The American Journal of Psychiatry*
Manuscript ID	AJP-17-07-0825.R1
Manuscript Type:	Article
Date Submitted by the Author:	n/a

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Keywords:	Adolescents - AJP0100, Brain Imaging Techniques - AJP0068, Outcome Studies - AJP0092

Title page

Word count: 3493

Tables: 3 (+6 in Supplemental Data)

Figures: 2 (+1 in Supplemental Data)

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Previous presentations

Results from this study were presented at the Society of Biological Psychiatry 72nd Annual Scientific Convention, SOBP, San Diego, CA, USA, May 17-20, 2017 and at the 17th International ESCAP Congress 2017, July 9-11, Geneva, Switzerland.

Location of work: INSERM, Research Unit UMR 1000, Research unit "Neuroimaging and Psychiatry", Paris; University Paris-Sud, University Paris-Saclay, Gif-sur-Yvette ; University Paris Descartes Paris; AP-HP, Western Paris University Hospital group, Department of Psychiatry, Paris; AP-HP, Department of Adolescent Psychopathology and Medicine, Maison de Solenn, Cochin Hospital, Paris; the Center for Neuroimaging Research (CENIR), Brain & Spine Institute, Paris; and the Psychiatry Department 91G16, Orsay Hospital, France; the Department of Social and Health Care, Psychosocial Services Adolescent Outpatient Clinic Kauppakatu 14, Lahti, Finland; the Department of Cognitive and Clinical Neuroscience, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Square J5, Mannheim, Germany; the Department of Child and Adolescent Psychiatry Psychosomatics and Psychotherapy, Campus CharitéMitte, Charité-Universitätsmedizin Berlin, Germany; the Department of Child and Adolescent Psychiatry, Institute of Psychiatry, Psychology & Neuroscience, King's College London,UK; the University Medical Centre Hamburg-Eppendorf, House W34, 3.OG, Hamburg, Germany; INSERM, Research Unit U952, UPMC, Quai Saint-Bernard, Paris; CNRS, Research Unit UMR 8194, 45 rue des Saints Pères, Paris, France; the Department of Child and Adolescent Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Square J5, Mannheim, Germany; the Centre for Neuroimaging Sciences, Institute of Psychiatry, Psychology & Neuroscience, King's College London,UK; Discipline of Psychiatry, School of Medicine and Trinity College Institute of Neurosciences, Trinity College, Dublin, Ireland; Physikalisch-Technische Bundesanstalt, Abbestr. 2 - 12, Berlin, Germany; the Medical Research Council - Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, Psychology & Neuroscience, King's College London; the Department of Psychological Medicine and Psychiatry, Institute of Psychiatry, Psychology & Neuroscience, King's College London, UK; the Department of Psychiatry, Université de Montréal, CHU Ste Justine Hospital, QC, Canada; Neurospin, Commissariat à l'Energie Atomique, CEA-Saclay Center, Paris, France; the Department of Psychiatry and Psychotherapy, University Medical Center Hamburg-Eppendorf, Martinistr. 52, 20246, Hamburg, Germany; the Departments of Psychiatry and Psychology, University of Vermont, 05405 Burlington, Vermont, USA; Sir Peter Mansfield Imaging Centre School of Physics and Astronomy, University of Nottingham, University Park, Nottingham,UK; the Department of Psychiatry and Psychotherapy, Campus CharitéMitte, Charité-Universitätsmedizin Berlin, Charitéplatz 1, Berlin, Germany; the Rotman Research Institute, University of Toronto, Canada; the Department of

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Disclosures and acknowledgments

Robert Goodman is the owner of Youthinmind, Ltd., which provides no-cost and low-cost software and websites related to the Development and Well-Being Assessment and the Strengths-and-Difficulties-Questionnaire. Gareth Barker received honoraria for teaching from General Electric Healthcare and acted as consultant of IXICO, and was granted US patent 2015-0123662A1 during the course of this work. Dr Stringaris receives royalties from the University of Cambridge for his book. Dr. Paillère-Martinot received compensation from Janssen-Cilag company for CME activities. Dr. Banaschewski served in an advisory or consultancy role for Actelion, Hexal Pharma, Lilly, Medice, Novartis, Oxford outcomes, PCM scientific, Shire and Viforpharma. He received conference support or speaker's fee by Medice, Novartis and Shire. He is/has been involved in clinical trials conducted by Shire & Viforpharma. He received royalities from Hogrefe, Kohlhammer, CIP Medien, Oxford University Press. Jürgen Gallinat has received research funding from the German Federal Ministry of Education and Research, German Science Foundation, AstraZeneca, Eli Lilly & Co, Janssen-Cilag, Bristol-Myers Squibb and speaker fees from AstraZeneca, Janssen-Cilag, and Bristol-Myers Squibb. All the other authors declare no competing interests.

Pascal Zille, research engineer, INSERM, Paris, France, and Sonia Martinot, student at Centrale-Supélec engineering School, Gif-sur-Yvette, France, are acknowledged for their contribution to machine-learning analyses.

Grant support

This work received support from the European Union-funded FP6 Integrated Project IMAGEN (LSHM-CT- 2007-037286), the FP7 project IMAGEMEND and the Innovative Medicine Initiative Project EU-AIMS (115300-2), a Medical Research Council Programme Grant (93558), the Swedish funding agency FORMAS, the Wellcome Trust (University of Cambridge), the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London, the Department of Health UK, the Bundesministerium für Bildung und Forschung (BMBF grants 01GS08152; 01EV0711; eMED SysAlc01ZX1311A; Forschungsnetz AERIAL), the French funding agency ANR (ANR-12-SAMA-0004), an Eranet – Neuron grant (project AF12-NEUR0008-01 - WM2NA), the Assistance-Publique-Hôpitaux-de-Paris and INSERM (interface grant), Paris-Descartes-University (collaborative-project-2010), Paris-Sud-University (IDEX-2012), the Fondation de France, the Fondation pour la Recherche Médicale, and the Mission Interministérielle de Lutte-contre-les-Drogues-et-les-Conduites-Addictives (MILDECA).

Abstract

Objective: White matter microstructure alterations have recently been associated with adolescence depressive episodes, but it is unknown whether they predate depression. We investigated whether subthreshold-depression in adolescence is associated with white matter microstructure variations and whether they relate to depression outcome.

Method: Adolescents with subthreshold-depression (n=96) and healthy controls (n=336), drawn from a community-based cohort, were compared using diffusion tensor imaging and whole-brain tract-based spatial statistics (TBSS) at age 14 to assess white matter microstructure. They were followed-up at age 16 to assess depression. Probabilistic tractography was used to reconstruct white matter streamlines from the TBSS analysis resulting regions, and along bundles implicated in emotion regulation, the uncinate fasciculus and the cingulum. We searched for mediating effects of white matter microstructure on the relationship between baseline subthreshold-depression and depression at follow-up, and then explored the specificity of the findings.

Results: Lower fractional anisotropy (FA) and higher radial diffusivity were found in the anterior corpus callosum in the adolescents with subthreshold-depression. Tractography analysis showed that they also had lower FA in the right cingulum streamlines, along with lower FA and higher mean diffusivity in tracts connecting the corpus callosum to the anterior cingulate cortex. The relation between baseline subthreshold-depression and follow-up depression was mediated by FA values in the latter tracts, and lower FA values in those tracts distinctively predicted higher individual risk for depression.

Conclusions: Early FA variations in tracts projecting from the corpus callosum to the anterior cingulate cortex might denote higher risk of transition to depression in adolescents.

Text

Introduction

First episodes of major depression have been associated with changes in white matter diffusion tensor imaging-derived indices in adolescents, mainly in the corpus callosum, the cingulum and the uncinate bundles (1-5). Evidence for white matter tract alterations in the frontal-thalamic-striatal circuit and in the default mode network nodes has also been found (6). Although the involvement of white matter alterations in depressive episodes is recognized, it is still unclear whether the alterations predate the onset of the disease, indicating vulnerability, or whether they indicate ongoing pathology. Studies in subjects at familial risk for depression have addressed this issue and found that white matter microstructure alterations might indicate genetic risk for affective disorders (7).

Subthreshold-depression in adolescence is another at-risk condition for depressive disorders, with an estimated risk of escalation to depression of 67% (8). It has been defined as an episode of clinically relevant depressive symptoms not meeting enough criteria for a diagnosis of a major depressive episode, either in number of symptoms, duration, or impact on functioning (9). In adolescence, subthreshold-depression has a high lifetime prevalence, ranging between 20 and 30% (9-10).

We recently reported that adolescents with subthreshold-depression had lower white matter volumes in the internal capsule, a region involved in frontal striatal networks, and in the forceps minor and the cingulum (11) that are part of the default mode network. Also, they had lower gray matter volumes in the medial prefrontal cortex and the striatum, and displayed functional striatal deficits when anticipating reward (12).

In the present study, we hypothesized that adolescents with subthreshold-depression would also present white matter microstructure differences within the frontal striatal-limbic and default mode networks, both implicated in depression.

To investigate white matter microstructure, we used tract-based spatial statistics (TBSS) to search for regional differences in diffusion tensor imaging (DTI)-derived indices within white matter tracts. We then performed probabilistic diffusion tractography to explore cortical projections from the region(s) identified in the TBSS approach (13), and to assess tractography-derived measures within the

cingulum and the uncinate fasciculus, two bundles reported altered in adolescent depression (2,3). Finally, based on the previous assumptions, we investigated whether the observed differences in DTI-derived indices would mediate the relationship between subthreshold-depression at age 14 and the occurrence of depression at age 16.

Method

Participants

The study was approved by the ethics committees in all participating institutions. Written informed assent and consent were obtained from all adolescents and their parents respectively, after complete description of the study.

Neuroimaging and clinical data were obtained from a large sample of community adolescents from eight sites in four European countries recruited in middle schools around age 14 (www.imagen-europe.com). A detailed description of recruitment and assessment procedures, with exclusion and inclusion criteria, has been published elsewhere (14). Notably, any obvious psychopathology at baseline (e.g. bipolar disorder, schizophrenia, or major neuro-developmental disorders) constituted non-inclusion criteria.

Baseline assessment

The adolescent psychiatric symptoms were assessed with the Development and Well-Being Assessment (DAWBA, www.dawba.com), a self-administered diagnostic questionnaire consisting of open and closed questions (15). The DAWBA provides computer-generated probabilities of having DSM-IV diagnoses, the DAWBA bands, and definitive diagnoses generated by clinical raters. Notably, the open comments from participants and their families provided in the DAWBA were taken into account to assess potential past history.

The assessment battery was self-administered both in participants' homes and at the neuroimaging facilities using Psytools software (Delosis Ltd, London, UK), via its internet-based platform (14). Substance use was reported using the Alcohol Use Disorders Identification Test (AUDIT) (16) and the European School Survey Project on Alcohol and Drugs (ESPAD, www.espad.org). Other assessments

included personality dimensions, using the Substance Use Risk Profile Scale (17), and the Neuroticism-Extraversion-Openness Five-Factor Inventory (18), handedness, pubertal status using the Pubertal Development Scale questionnaire (19), parental psychiatric history (14, Supplemental Data), and life-events using the Life-Events Questionnaire (20). Participants with any psychiatric diagnosis (e.g. major depressive disorder, bipolar disorder, attention deficit hyperactivity disorder…), any history of life-time drug use, or any symptoms of alcohol abuse or dependence (AUDIT score >4) were excluded from the present study, as those disorders have neural correlates that would have biased our neuroimaging investigation. No participant or their parent reported being prescribed antidepressants, mood stabilizers, anxiolytics, antipsychotics or hypnotics.

Adolescents were included in the subthreshold-depression group if, in the last 4 weeks, they had experienced, at least 3 depressive symptoms including at least one core symptom (abnormally depressed, irritable mood, or loss of interest) and 2 or more other DSM-IV depressive symptoms, without fulfilling criteria for a major depressive episode in terms of duration, symptom number, or significant impact on functioning (11,12). The control group matched for sex with the subthreshold-depression group included adolescents with less than 3 symptoms of depression and a probability of having a diagnosis of major depression of less than 0.1% according to the DAWBA. Participants with less than 3 symptoms constitute the standard in the IMAGEN community-based sample of adolescents with a prevalence of 85% (11).

At baseline, 2223 IMAGEN adolescents completed the DAWBA (SF1). Thirty-seven (1.66%) had depression and 301 (13.54%) had subthreshold-depression. After considering exclusion criteria, the present study included 96 subthreshold-depression and 336 control adolescents.
Eighty-one percent of the subthreshold-depression adolescents, and 73% of the controls, had participated in our previous report on regional brain volumes (11).

Follow-up assessment

Two years after baseline, at age 16, participants were followed up using the same web-based questionnaires, but they did not undergo neuroimaging. The presence of a depression diagnosis was

assessed using the DAWBA. Among the 1717 IMAGEN participants who completed the DAWBA, 42 (2.45%) had depression and 214 (12.46%) had subthreshold-depression.

Follow-up of the initial subthreshold-depression and control groups retained 84.4% (n=81) and 84.5% (n=284) of the baseline participants, respectively ($\chi^2(1, N=432)=0.00$, p=1) (Table 1). Lost-to-follow-up participants were more likely to be non-Caucasian than those followed-up (Supplemental Data, Methods).

Diffusion MRI acquisition and preprocessing

Diffusion images were acquired on 3 Tesla scanners using an Echo Planar imaging sequence adapted to tensor measurements and tractography analysis (Supplemental data).

Diffusion data preprocessing was performed using FMRIB Software Library (FSL) (www.fmrib.ox.ac.uk/fsl) (21) to obtain tensor-based parameters such as fractional anisotropy (FA), mean diffusivity (MD), Axial Diffusivity (AD) and Radial Diffusivity (RD) (Supplemental data).

Tract-Based Spatial Statistic

Voxel-wise statistical analysis of the FA data was carried out using TBSS, part of FSL (22). All participants' FA data were aligned into a common space using the nonlinear registration tool FNIRT (23,24). Next, the mean FA image was created and thinned to create a mean FA skeleton, which represents the centers of all tracts common to the group. This skeleton was then thresholded to FA>0.2 to keep only the main tracts. Each adolescent's aligned FA, MD, AD and RD data were then projected onto the skeleton and the resulting data fed into voxel-wise cross-individual statistics. All control and subthreshold-depression adolescent DTI images passed a quality control in order to discard altered images with head movement, poor tensor computation or defective spatial normalization (SF1).

Probabilistic diffusion tractography

The result from the between-group comparison of FA was used to create a seed region to perform probabilistic diffusion tractography. The warpfields of nonlinear registration and their inverses were used for the translation between the original space and the Montreal Neurological Institute (MNI)-152 standard space. Briefly, diffusion data were used to estimate a probability distribution function of fiber

direction allowing modeling multiple fiber orientations at each voxel. Tractography then proceeds by drawing 5000 streamlines through these probability distributions from each seed voxel to target 45 cortical and 15 subcortical regions based on the Harvard-Oxford atlases. For each target region, we extracted the number of samples reaching it from all seed voxels. Only pathways demonstrating a high number of streamlines to the target (mean samples >1000) were considered for the statistical analyses. In addition, we used the same probabilistic tractography algorithm to reconstruct, in each participant, the uncinate fasciculus and the cingulum bundle based on seed and target region described by Wakana and collaborators (25). A mean streamline map was created for each pathway of interest and thresholded to 1000 samples to create a mask. Mean samples, FA, MD, RD and AD were computed within each mask for each participant.

Statistical analysis

Cross-sectional analyses

Voxel-wise group comparisons on FA and MD maps were tested in the framework of the general linear model using a randomization-based method (5,000 permutations). AD and RD were compared when differences in FA values were observed. Analyses were conducted including FA and MD as the dependent variables, group (subthreshold-depression or control) and sex as main factors, and group-by-sex interaction to explore a potential sex effect. If the interaction term was not statistically significant, only group was considered as main factor, and sex was added as confounding factor. Age and DTI acquisition type (i.e. scanner manufacturers and/or software level to account for inter-scanner variance) were entered as confounding covariates. Statistical thresholds were set at $p<0.05$ family-wise error corrected and Threshold-Free Cluster Enhancement corrected (26). The ICBM-DTI-81 white matter labels atlas (27) and the Johns Hopkins University tractography atlas (25) were used within FSLview to locate the tracts that displayed significant differences.

Group comparisons for socio-demographic, clinical characteristics, global diffusion values and regional tractography measures, were performed within the framework of the general linear model using R software (http://cran.r-project.org). As in voxel-based analyses, group-by-sex interactions were tested before considering group as main factor and sex as confounding factor. Age and centers were entered as confounding covariates. AD and RD were compared when differences in FA values were observed.

Longitudinal analyses

Binomial logistic regressions were used to examine the association between subthreshold-depression at age 14, entered as predictor of interest, and depression at age 16, entered as the dependent variable. Age, sex and center were entered as confounding covariates.

Causal mediation analyses were then conducted to determine whether FA and MD measures identified in TBSS and tractography analyses could mediate the relation between subthreshold-depression at age 14 and depression at age 16. They were performed with a validated algorithm using a set of general linear models to derive the mediation and direct effects from the total effect (28). Depression at age 16 was entered as dependent factor, and group (subthreshold-depression or control) as independent factor within a logistic regression model. FA and MD values were entered as mediator variable. Age, sex and DTI acquisition type were entered as confounding covariates. This mediation model was performed using 5000 Monte Carlo draws for nonparametric bootstrap. In causal mediation analysis, a significant mediating effect is defined as a 95% confidence interval that does not include zero.

General linear models were used to examine the associations between the clinical characteristics that were associated with subthreshold-depression at age 14, and a diagnosis of depression at age 16. We also examined the associations between these clinical characteristics and FA and MD measures that we identified in TBSS and tractography analyses.

Post-hoc analyses

The specificity of the neuroimaging and clinical findings was investigated in the study sample (n=365), and the reproducibility and specificity of the findings were explored in a distinct sample of 686 healthy adolescents at baseline (Supplemental Data, Method). For those analyses, the probabilities of having a diagnosis, measured with the DAWBA bands, were used as risk levels (ST3, ST6).

Finally, a machine-learning analysis was performed in the study sample, aiming at individually predicting depression diagnosis at age 16, based on FA values in tracts spanning from the anterior corpus callosum cluster to the anterior cingulate cortex at age 14 (Supplemental Data, Method).

Results

Participant socio-demographic and clinical characteristics

Adolescents with subthreshold-depression had significantly higher scores on dimensions of Hopelessness, Anxiety-Sensitivity, Impulsivity, and Neuroticism, along with lower scores on Extraversion, Agreeableness, and Conscientiousness (Table 1). They also had a higher level of negative life events, were more likely to have at least one non-white parent and to have more familial financial difficulties. No sex-by-group interaction was found.

At follow-up, adolescents with subthreshold-depression similarly differed from controls except for anxiety-sensitivity (Table 1). In addition, they reported significantly more self-harm since baseline and were more likely to have subthreshold or full depression, and alcohol abuse.

DTI whole brain analyses

Subthreshold-depression adolescents (vs. controls) globally had lower FA values and higher RD values (Table 1). No sex-by-group interaction was found regarding DTI global measures.

Compared with controls, subthreshold-depression adolescents had lower FA and higher RD within the genu and the anterior body of the corpus callosum, including the forceps minor and anterior thalamic radiation (Table 2, Figure 1A).

Tractography analysis

Using the anterior corpus callosum cluster as a seed region for probabilistic tractography, we found a high number of cortical streamlines (mean samples > 1000) reaching the anterior cingulate cortex (Brodmann areas (BA) 24, 25, 32, 33), middle frontal (BA 9, 10, 46), frontal pole (BA 9, 10, 11, 12), superior frontal (BA 4, 6, 8), and paracingulate regions (BA 24 and 32, corresponding to the dorsal part of the anterior cingulate cortex) (Figure 1B). There was no between-group difference in the number of samples to any of these target regions (ST1).

After correction for multiple comparisons, lower FA and higher MD and RD were found in adolescents with subthreshold-depression within tracts reconstructed between the anterior corpus callosum cluster and the anterior cingulate cortex (Table 2) that included the forceps minor, cingulum, anterior thalamic radiation, superior longitudinal fasciculus and inferior fronto-occipital fasciculus.

In the tractography analyses of the cingulum and uncinate fasciculus based on *a priori* hypotheses, the adolescents with subthreshold-depression had a higher number of samples, lower FA, and no difference in MD, in the right cingulum (Table 2). They also had higher RD in this region with no difference in AD.

No sex-by-group interaction effects were found in any of the voxel-based or the tractography-based analyses.

Longitudinal analyses

Subthreshold-depression at age 14 was associated with an increased risk of depression diagnosis at age 16 (Odd ratio= 3.96; 95% Confidence interval= [1.27; 12.38]).

Higher scores of neuroticism and hopelessness at age 14 were also associated with an increased risk of depression diagnosis at age 16 (β=6.61, t=1.88, p<0.001 and β=3.43, t=0.78, p<0.001 respectively), but not with any DTI measure (ST2).

Causal mediation analyses showed that lower FA values in tracts spanning from the anterior corpus callosum cluster to the anterior cingulate cortex accounted for 21% (p=0.01) of the total variance in the relationship between subthreshold-depression at baseline and depression at age 16. At trend level, higher MD values also had a mediation effect (Figure 2A; Table 3).

Post-hoc analyses

In the study sample, subthreshold-depression at age 14 was associated with higher risk at age 16 for various DSM-IV disorders (Supplemental Data, Results). Lower FA values in tracts spanning from the anterior corpus callosum cluster to the anterior cingulate cortex at age 14 predicted higher risk for depression, but not for any other diagnosis, at age 16, either in the study sample or in the distinct sample of 686 adolescents (Supplemental Data, Results).

In contrast, in the study sample, higher scores of neuroticism and hopelessness at age 14 mediated the association between subthreshold-depression at age 14 and risk for both depression and other diagnoses at age 16 (Supplemental Data, Results, ST4, ST5).

Finally, individual prediction of depression diagnosis at age 16, using FA values in tracts spanning from the anterior corpus callosum cluster to the anterior cingulate cortex at age 14, resulted in an *Area Under the Curve* (AUC) of 0.75 with a $p<0.001$ (Figure 2C), denoting the likelihood of FA values in those tracts in predicting the onset of depression for an individual adolescent.

Discussion

Regional variations in white matter microstructure were identified for the first time in 14-year-old community adolescents with subthreshold-depression and no familial risk for depression. These differences involved the anterior body and genu of the corpus callosum. Tractography analyses revealed variations in the right cingulum and within fibers between the anterior corpus callosum and the anterior cingulate cortex. Lower FA values in those prefrontal tracts mediated transition to depression at age 16. Furthermore, in a larger sample of healthy adolescents, we found that lower FA in these fibers indicated risk for depression but not for other diagnoses.

Alterations in similar tracts have previously been reported in depressed adolescents and adults (1-4, 29), and in adolescents at familial risk (7).

The corpus callosum anterior part, frequently implicated in adolescent depression (2-4), connects to the anterior cingulate cortex, a core region of the pathophysiology of depression, which volume we previously reported smaller, and was found to mediate depression outcome two years later, in the same participants (11) (Figure 2B).

The present tractography analysis also revealed white matter microstructural variations in the cingulum bundle in the adolescents with subthreshold-depression, consistently with our previous report that they had smaller white matter volume than the controls in the right cingulum (11). The cingulum contains fibers connecting the cingulate, prefrontal cortices, and temporal limbic regions, and is involved in functional connectivity of the default mode network (33).

In the present study, lower FA values were also observed in the right anterior thalamic radiation that runs in close proximity to the supero-lateral branch of the medial forebrain bundle. Both tracts may be difficult to distinguish and are not differentiated in the atlas used in this study (25,31). Converging both in the anterior limb of the internal capsule and in the prefrontal cortex (31), they might be differentially

involved in the pathophysiology of depression. The anterior thalamic radiation would convey negative affective states, such as sadness, whereas the supero-lateral branch of the medial forebrain bundle would be involved in reward-seeking and appetitive motivation (31), as it connects core regions of the reward system. Also, lower FA in this tract has been associated with higher anhedonia (32). In previous studies in almost the same group of 14 years-old adolescents with subthreshold-depression, we consistently found functional striatal deficits when anticipating reward (12), and smaller gray matter volumes in the caudates (11). Our findings also indicate that FA measures at age 14, in tracts spanning from the anterior corpus callosum cluster to the anterior cingulate cortex, hold a significant individual predictive value for later depression, with a fair accuracy. Altogether, these complementary findings suggest that a particular developmental pathophysiology involving these white matter tracts and adjacent gray matter regions, might account for the vulnerability to develop depression.

White matter changes have also been associated with other psychiatric disorders in adolescents, notably generalized anxiety disorder and attention-deficit/hyperactivity disorder (34,35). Herein, subthreshold-depression at age 14 was also associated with risk for such disorders at follow-up, in line with the literature (36), and higher neuroticism and hopelessness scores at age 14 were found to mediate the association between subthreshold-depression at age 14 and risk for both depression and other diagnoses at age 16. However, lower FA values did not predict higher risk for any other diagnosis than depression at follow-up, neither in the study sample, nor in the distinct sample of 686 adolescents healthy at baseline. Thus, our imaging findings appear specific of increased risk for depression.

In line with the literature, the subthreshold-depression participants in this study had higher scores on neuroticism and hopelessness, and negative life-events. It has been suggested that such characteristics may alter individual sensitivity to stressful events and increase the risk of depression (37). In the immature, adolescent brain, these factors might interact with hormonal and maturational changes. Indeed, adolescence is a critical period for white matter maturation, as white matter volumes and FA values show a clear increase throughout adolescence, particularly in some tracts involved in emotion regulation (38,39). Thus, in the present study, lower FA might indicate white matter microstructure alteration, as well as delayed white matter maturation.

A first limitation of the study is inherent to DTI, which is currently the only method capable of mapping the fiber architecture of tissue in vivo (40), but whose results should be interpreted with caution, as it

does not allow the uncovering of the mechanisms underlying variations in diffusion tensor measures. Second, due to inclusion/exclusion criteria and cultural differences, the proportions of subthreshold-depression and control adolescents differed between sites. For instance, fewer adolescents were eligible as controls in London. Even though we controlled for DTI acquisition type in the analyses, the between-scanner variability in our measurements may have underpowered the detection of the effects of interest and needs to be considered as a limitation of our study. Third, as discussed elsewhere (11), the self-administered symptom rating could have biased the diagnostic ratings. However, the rate of IMAGEN participants with subthreshold-depression, and the rate of participants who transitioned to depression are consistent with the rates reported in the literature. Finally, considering follow-up evaluation, DTI was not repeated at age 16 and only psychometric data were available.

In summary, variations in DTI measures in brain regions involved in emotion regulation were detected in 14-year-old community adolescents with subthreshold-depression and no familial risk. The results suggest that these adolescents might have delayed white matter maturation or early alterations in tracts spanning from the anterior corpus callosum to the anterior cingulate cortex, which in turn might denote high risk for transition to depression.

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Figure Legends

Figure 1:

A. Regions with lower fractional anisotropy in 96 adolescents with subthreshold-depression versus 336 controls. Left: transversal, Middle, coronal, and Right: sagittal slices. Green indicates white matter skeleton. FA images are displayed using the "tbss_fill" script, which allows better visualization of the regions with significant between-group differences. FWE, Family-wise error corrected; TFCE, Threshold-free cluster enhancement; R, Right. Results are superimposed on the average T1-weighted MRI of the adolescent brains of the IMAGEN database.

B. Probabilistic diffusion tractography from the anterior corpus callosum seed thresholded to 1000 samples. Middle frontal gyrus (dark blue), frontal pole (light blue), superior frontal gyrus (green), paracingulate gyrus (pink), anterior cingulate gyrus (brown) from the Harvard-Oxford atlases.
Results are superimposed on the average T1-weighted MRI of the adolescent brains of the IMAGEN database.

Figure 2:

A. Mediation of FA values in tracts spanning from the anterior corpus callosum cluster to the anterior cingulate on the relationship between subthreshold-depression at age 14 and depression at age 16, using causal mediation analysis.

B. Three-dimensional representation (http://brainvisa.info) of mediation results projected onto a T1-weighted MRI scan of an adolescent brain from the IMAGEN database; Green: anterior cingulate cortex, Blue: probabilistic diffusion tractography from the anterior corpus callosum white matter cluster to the anterior cingulate cortex, Red: medial prefrontal cortex grey matter cluster, which lower volume mediated the relation between subthreshold-depression at age 14 and depression at age 16 in Vulser et al., 2015 (11).

C. Receiver Operating Characteristic (ROC) curve illustrating individual prediction of depression diagnosis at age 16 from fractional anisotropy values in tracts spanning from the anterior corpus callosum cluster to the anterior cingulate cortex at age 14. Study sample n=365 adolescents; AUC: area under the curve can vary between 0 - 1; an uninformative classifier would yield 0.5

Table 1: Clinical characteristics in adolescents with subthreshold-depression compared with controls at baseline and follow-up

Characteristics	Baseline						Follow-up					
	Subthreshold-depression N=96		Controls N=336		Test	p-value	Subthreshold-depression N=81		Controls N=284		Test	p-value
	Mean	**SD**	**Mean**	**SD**	**F(1,422)**		**Mean**	**SD**	**Mean**	**SD**	**F(1,355)**	
Age (years)	14.47	0.38	14.41	0.40	2.26	0.13	16.47	0.49	16.43	0.52	0.42	0.52
	Mean	**SD**	**Mean**	**SD**	**F(1,421)**		**Mean**	**SD**	**Mean**	**SD**	**F(1,354)**	
Pubertal Development Scale (0-5)	3.03	0.52	2.95	0.54	0.90	0.34	3.45	0.40	3.41	0.40	0.34	0.56
	N	**%**	**N**	**%**	**χ^2(1, N=432)**		**N**	**%**	**N**	**%**	**χ^2(1, N=365)**	
Sex (females)	62	64.58	217	64.58	0.00	1	52	64.20	182	64.08	0.00	1
Handedness (right-handed)	80	83.33	298	88.69	1.50	0.22	67	82.72	253	89.08	1.81	0.18
Parental history of depression	8	8.33	24	7.14	0.03	0.86	6	7.41	19	6.69	0.00	0.98
Any parental psychiatric history	15	15.63	46	13.69	0.12	0.73	12	13.38	38	14.81	0.02	0.88
Non-Caucasian ethnicity	21	21.88	27	8.04	13.11	**<0.001**	13	16.05	68	5.28	8.85	**0.003**
Adverse childhood experience[a]	12	12.50	30	8.93	0.77	0.38	4	4.94	28	9.86	1.34	0.25
Familial financial difficulties	36	37.50	82	24.40	5.81	**0.02**	29	35.80	73	25.70	2.71	0.10

Subthreshold-depression at age 16	_	_	_	_	_	_	22	27.16	29	10.21	13.69	**<0.001**
Depression at age 16	_	_	_	_	_	_	8	9.88	7	2.46	7.01	**0.008**
Self-harm since age 14	_	_	_	_	_	_	24	29.63	24	8.51	22.65	**<0.001**
Anxiety disorder at age 16[b]	_	_	_	_	_	_	1	1.23	5	1.76	_	1
Externalizing disorder at age 16[c]	_	_	_	_	_	_	1	1.23	1	0.35	_	0.40
Psychosis at age 16	_	_	_	_	_	_	0	0	0	0	_	_
Mania at age 16	_	_	_	_	_	_	0	0	1	0.35	_	1
Monthly cannabis use at age 16	_	_	_	_	_	_	6	7.41	18	6.33	0.01	0.93
Alcohol abuse at age 16[d]	_	_	_	_	_	_	15	23.81	26	12.38	4.10	**0.04**
	Mean	**SD**	**Mean**	**SD**	**U**	**p-value**	**Mean**	**SD**	**Mean**	**SD**	**U**	**p-value**
Five-Factor Inventory												
Neuroticism	29.11	7.46	20.97	6.76	6802	**<0.001**	27.16	6.42	20.67	7.28	5483	**<0.001**
Extraversion	27.83	6.68	30.75	5.30	19924	**<0.001**	27.58	6.33	30.26	5.59	13961	**<0.001**
Openness	26.47	6.55	26.30	5.57	16070	0.96	28.21	6.47	27.46	6.03	10610	0.47
Agreeableness	26.44	5.67	30.91	5.07	23380	**<0.001**	27.31	5.11	31.03	5.55	15633	**<0.001**
Conscientiousness	26.57	7.35	29.19	6.45	19572	**0.001**	26.73	7.62	29.65	6.79	13635	**0.003**
Substance Use Risk Profile Scale												
Hopelessness	14.89	3.93	12.42	2.54	10079	**<0.001**	14.18	3.98	12.44	3.06	8174	**<0.001**

Anxiety-Sensitivity	11.86	2.43	11.05	2.24	13320	**0.009**	11.35	2.53	10.95	2.38	9920	0.14
Impulsivity	13.04	2.37	11.42	2.01	9924	**<0.001**	12.03	2.06	10.95	2.11	7815	**<0.001**
Sensation-seeking	16.21	3.03	15.58	3.13	14362	0.10	13.83	2.61	13.80	2.80	11306	0.82
Life-events score (-78; +78)	-1.90	4.75	-0.23	4.44	19319	**0.003**	0.61	5.45	2.11	4.33	12526	**0.045**

SD, standard deviation; Life-Events score: Life-events Questionnaire total score; χ^2, chi-square value; F, ANOVA's F-value; U, Mann–Whitney–Wilcoxon U value; [a] At least one lifetime adverse experience at baseline, adverse experience since baseline at follow-up; [b] Anxiety disorders include: Social Phobia, Panic disorder, Agoraphobia, Generalized Anxiety Disorder, Post-traumatic Stress Disorder and obsessive-compulsive disorder; [c] Externalizing disorders include: Attention Deficit Hyperactivity Disorder, Oppositional-Defiant Disorder Oppositional-Defiant Disorder and Conduct Disorder; [d] Defined as AUDIT≥7; **bold** figures indicate significant results at $p<0.05$.

Table 2: Neuroimaging characteristics in adolescents with subthreshold-depression compared with controls at baseline

Characteristics	Subthreshold-depression N=96		Controls N=336		Test	
	Mean	**SD**	**Mean**	**SD**		**p-value**
DTI global measures					**F(1,425)**	
Global FA values (x10^{-1})	4.47	0.17	4.50	0.20	3.96	**0.047**
Global MD values (x10^{-4})	7.35	0.24	7.26	0.20	2.38	0.12
Global AD values (x10^{-3})	1.13	0.34	1.12	0.29	0.28	0.60
Global RD values (x10^{-4})	5.40	0.23	5.31	0.22	3.89	**0.049**
DTI measures within the cluster region identified in voxel-wise comparison [a]						
FA values (x10^{-1})	6.23	0.38	6.39	0.39	19.41	**<0.001**
RD values (x10^{-4})	5.38	0.24	5.20	0.24	30.84	**<0.001**
Tractography analyses						**p-value** [b]
Tracts spanning from the anterior corpus callosum cluster to the anterior cingulate cortex						
FA values (x10^{-1})	4.51	0.31	4.60	0.34	10.06	**0.01**
MD values (x10^{-4})	8.04	0.46	7.85	0.36	8.56	**0.03**
AD values (x10^{-3})	1.24	0.06	1.22	0.05	1.58	0.21
RD values (x10^{-4})	5.85	0.49	5.66	0.42	10.93	**0.001**
Right cingulum bundle						
FA values (x10^{-1})	3.45	0.34	3.59	0.39	9.13	**0.03**
MD values (x10^{-4})	8.16	0.54	7.98	0.45	0.01	1
AD values (x10^{-3})	1.13	0.07	1.12	0.06	0.02	0.88
RD values (x10^{-4})	6.60	0.54	6.40	0.48	7.29	**0.007**
Left cingulum bundle						
FA values (x10^{-1})	3.71	0.39	3.80	0.41	5.17	0.24
MD values (x10^{-4})	8.24	0.54	8.08	0.45	2.39	1

Right uncinate bundle						
FA values ($x10^{-1}$)	3.99	0.39	3.99	0.35	0.02	1
MD values ($x10^{-4}$)	7.85	0.40	7.73	0.29	0.45	1
Left uncinate bundle						
FA values ($x10^{-1}$)	4.01	0.49	3.99	0.48	0.42	1
MD values ($x10^{-4}$)	8.01	0.47	7.88	0.29	0.12	1

SD, standard deviation; DTI, diffusion tensor imaging; FA, fractional anisotropy; MD, mean diffusivity; AD, Axial Diffusivity; RD, Radial Diffusivity (RD); FWE, Family-wise error corrected; TFCE, Threshold-free cluster enhancement; [a] cluster size k=234; peak-voxel MNI coordinates at x=14 mm, y=30 mm, z=15 mm; [b] p-value corrected for multiple comparisons; **bold** figures indicate significant results at $p<0.05$.

Table 3: Causal mediation analysis on the relationship between subthreshold-depression at age 14 and depression at age 16 with FA and MD values in tracts spanning from the anterior corpus callosum cluster to the anterior cingulate as mediator.

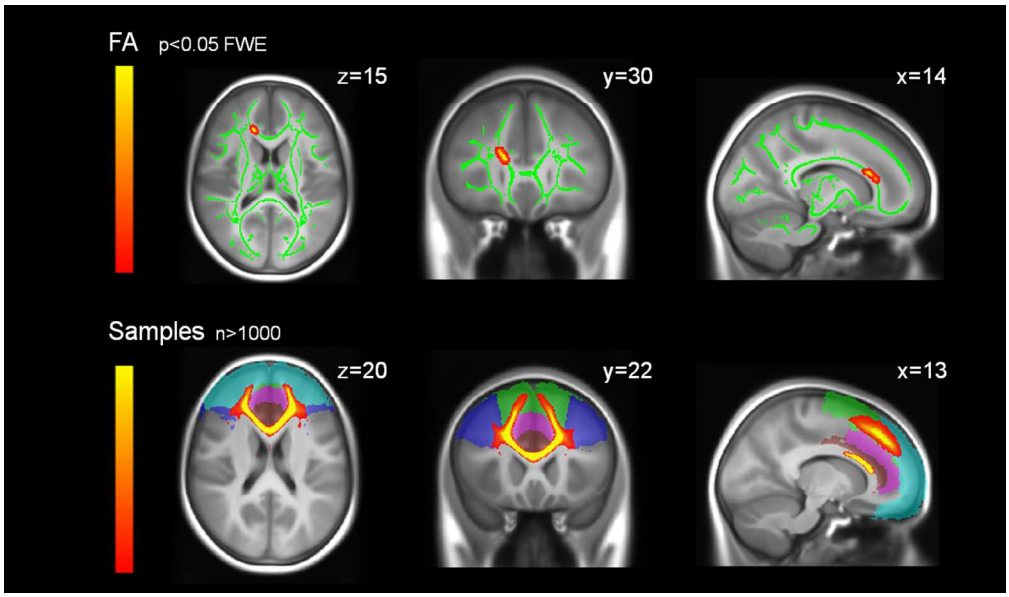
Mediator variable: FA values in tracts spanning from the anterior corpus callosum cluster to the anterior cingulate

Effect type	Point estimate	95% CI	p-value
Mediation effect (subthreshold-depression at 14 - FA- depression at 16)	0.018	[0.004; 0.044]	0.01
Direct effect (subthreshold-depression at 14 - depression at 16)	0.067	[-0.001; 0.162]	0.06
Total effect	0.085	[0.016; 0.184]	0.01
Proportion of total effect via mediation	0.206	[0.042; 0.902]	

Mediator variable: MD values in tracts spanning from the anterior corpus callosum cluster to the anterior cingulate

Effect type	Point estimate	95% CI	p-value
Mediation effect (subthreshold-depression at 14 - MD - depression at 16)	0.010	[0.000;0.027]	0.06
Direct effect (subthreshold-depression at 14 - depression at 16)	0.073	[0.008; 0.016]	0.02
Total effect	0.083	[0.016; 0.177]	0.01
Proportion of total effect via mediation	0.017	[-0.002; 0.512]	

FA: Fractional Anisotropy; Point estimate: estimate of the size of the effect; 95% CI; 95% confidence interval of the point estimate.



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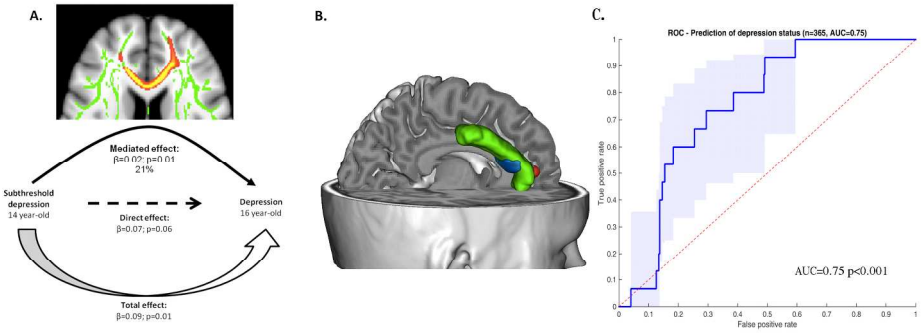

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