Data Supplement for Vulser et al., Early Variations in White Matter Microstructure and Depression Outcome in Adolescents With Subthreshold Depression. Am J Psychiatry (doi: 10.1176/appi.ajp.2018.17070825)

Method

Participants

Baseline assessment

Parental psychiatric history was assessed using a modified version of the Family Interview for Genetics Studies, the GEN (Genetic Screening and Family History of Psychiatric Disorders Interview) (1). Parental psychiatric history was self-reported by one of the parents. The assessed diagnoses were: major depressive disorder, schizophrenia, schizoaffective disorder, bipolar disorder, obsessive-compulsive disorder, anxiety disorder, eating disorder, alcohol problems, drug problems, and "other diagnosis".

FIGURE S1. Flow diagram of group selection
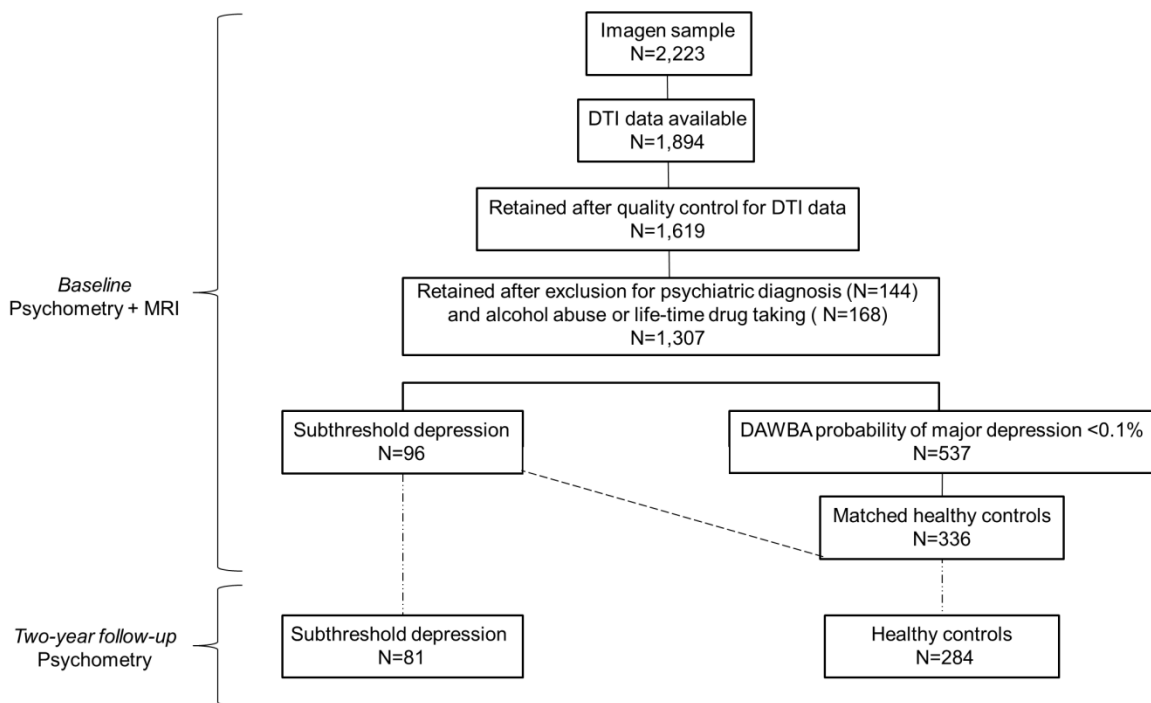
Follow-up assessment

Lost-to-follow-up participants did not differ from those followed-up in any characteristic, except that they were more likely to be non-Caucasian (n=20 (29.85%), versus n=28 (7.67%) followed-up; $\chi^2(1, N=432)=13.11$; $p<0.001$).

MRI data acquisition and preprocessing

Diffusion tensor images were obtained at age 14 on 3 Tesla scanners (Siemens; Philips; General Electric). The diffusion tensor images were acquired using an Echo Planar imaging sequence (4 b-value=0 s/mm2 and 32 diffusion encoding directions with b-value=1300 s/mm2; 60 oblique-axial slices (angulated parallel to the anterior commissure/ posterior commissure line); echo time ≈ 104 ms; 128x128 matrix; field of view 307x307mm; voxel size 2.4 x 2.4 x 2.4 mm), adapted to tensor measurements and tractography analysis. Where available, a peripherally gated sequence was used; when this was not possible, TR was set to 15s, approximately matching the effective TR of the gated scans.

Diffusion data preprocessing was performed using FMRIB Diffusion Toolbox (FDT) in FMRIB Software Library (FSL) (www.fmrib.ox.ac.uk/fsl) and consisted of affine registration to the first b=0 image for head motion and eddy currents correction, brain extraction using the Brain Extraction Tool (BET) (2), and voxel-wise diffusion tensor fitting to obtain images of fractional anisotropy (FA), mean diffusivity (MD), Axial Diffusivity (AD) and Radial Diffusivity (RD).

Post-hoc analyses

Specificity of the neuroimaging findings in the study sample

In order to explore the specificity of lower FA values in the tracts spanning from the anterior corpus callosum cluster to the anterior cingulate cortex, we first explored the association between lower FA values in those tracts at age 14 and risk for other diagnoses than depression at age 16.

Due to the small number of participants making a transition to a validated diagnosis of a full-blown disorder between ages 14 and 16, the probabilities of having a diagnosis, measured with the DAWBA bands, were used as risk levels. The DAWBA generates six levels of risk for each diagnosis: <0.1%, ~0.5%, ~3%, ~15%,

~50%, >70%. We used the DAWBA probabilities of having a DSM-IV diagnosis at age 16 to define risk for generalized anxiety disorder, social phobia, panic disorder, obsessive compulsive disorder, attention deficit hyperactivity disorder, oppositional-defiant disorder, and conduct disorders. A general probability of having "any DSM-IV diagnosis" at age 16 was also used.

We first examined the association between group (subthreshold-depression or control) and risk for psychiatric disorders. We used Poisson regression models with the 6 levels of risk for each of the 7 diagnoses at age 16 and for "any DSM-IV diagnosis" at age 16 as the outcomes in 8 separate models. Group at age 14 was entered as predictor of interest. Age, sex and centers were entered as confounding covariates.

Then, we examined the association between FA values and risk for psychiatric disorders at age 16 when an association with subthreshold-depression at age 14 had been found. We used Poisson regression models with the 6 levels of risk for the diagnosis at age 16. FA values at age 14 were entered as predictor of interest. Age, sex and DTI acquisition type were entered as confounding covariates.

Specificity of the clinical findings in the study sample

Based on the clinical findings. that higher scores of neuroticism and hopelessness at age 14 were associated with an increased risk for depression diagnosis at age 16, we examined whether higher scores for neuroticism and hopelessness at age 14 could mediate the association between subthreshold-depression at age 14 and depression at age 16.

Causal mediation analyses (3) were conducted using neuroticism or hopelessness score at age 14 as mediator variable in two separated models. Depression at age 16 was entered as dependent factor, and group (subthreshold-depression or control) as independent factor within a logistic regression model. Age, sex and center were entered as confounding covariates.

Then in order to explore the specificity of higher scores of neuroticism and hopelessness, we explored the association between these scores at age 14 and risk for other diagnoses than depression at age 16. We used Poisson regression models with the 6 levels of risk for "any DSM-IV diagnosis" at age 16 as the

outcome. Neuroticism or hopelessness scores at age 14 were entered as predictor of interest. Age, sex and centers were entered as confounding covariates.

If the association was significant, we then searched for a mediation effect of higher scores of neuroticism or hopelessness on the relationship between subthreshold-depression at age 14 and increased risk for any psychiatric disorder. Causal mediation analyses (3) were conducted using scores of neuroticism or hopelessness at age 14 as mediator variable in two separated models. The six probability levels of having "any DSM-IV diagnosis" at age 16 were entered as dependent factor within a Poisson regression model. Age, sex and center were entered as confounding covariates.

Reproducibility of the findings in a distinct sample (n=686)

Based on the main findings, we tested the complementary hypothesis that lower FA values in tracts spanning from the anterior corpus callosum cluster to the anterior cingulate cortex were associated with higher risk levels of depression at follow-up in a distinct sample of healthy adolescents at baseline.

The distinct sample of healthy adolescents drawn from the IMAGEN database had no overlap with the study sample (subthreshold-depression and control). At baseline, these adolescents had no validated psychiatric diagnosis, no life-time history of drug use, and no symptom of alcohol abuse or dependence (AUDIT score >4). No participant or their parent reported being prescribed antidepressants, mood stabilizers, anxiolytics, antipsychotics or hypnotics. After quality control for neuroimaging data the sample included 686 adolescents.

In this distinct sample, mean FA at age 14 was computed for each adolescent's FA image within a mask (mean samples > 1000) of tracts spanning between the anterior corpus callosum cluster to the anterior cingulate cortex derived from the original sample of 432 adolescents.

We used the DAWBA probabilities of having a diagnosis of depression at age 16 to define the risk level.

We used Poisson regression models with the 6 levels of risk for depression at age 16 as the outcome and FA values at age 14 as predictor of interest. Age, sex and DTI acquisition type were entered as confounding covariates.

Specificity of the neuroimaging findings in the distinct sample

We explored the specificity of lower FA values in other diagnoses than depression in the distinct sample of healthy adolescents at baseline.

We used Poisson regression models with the 6 levels of risk for each of the 7 diagnoses other than depression at age 16 and for "any DSM-IV diagnosis" at age 16 as the outcomes in 8 separate models. FA values in tracts spanning from the anterior corpus callosum cluster to the anterior cingulate cortex 14 were entered as predictor of interest. Age, sex and DTI acquisition type were entered as confounding covariates.

Machine Learning based on FA values derived from tractography in the study sample.

We performed a machine learning analysis aiming at 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. We used a leave-one-out cross-validation procedure where FA values were used as predictors of depression diagnosis at age 16. For each fold, a linear support vector machine classifier was trained on the training set, and then used to compute a classification score for the remaining subject from the test set. The overall predictive power is assessed by computing the *Area Under the Curve* (AUC) using depression diagnosis at age 16 and predicted scores across all folds. In order to assess the significance of such test statistic (i.e. the AUC value), we generated an empirical null distribution using permutations. We re-performed the whole leave-one-out analysis 5000 times where each time, depression diagnosis was randomly shuffled with respect to FA values. Then, the original observed AUC value was compared to this null distribution to obtain a p-value.

Results

Tractography analysis

TABLE S1. Results of the probabilistic tractography analyses in adolescents with subthreshold-depression compared with controls.

	Subthreshold depression N=96		Controls N=336		Test		Subthreshold depression N=96		Controls N=336		Test		Subthreshold depression N=96		Controls N=336		Test	
	Number of samples						Fractional Anisotropy						Mean Diffusivity (x10^{-4})					
	Mean	SD	Mean	SD	F(1,420)	p_c	Mean	SD	Mean	SD	F(1,420)	p_c	Mean	SD	Mean	SD	F(1,420)	p_c
Tractography analyses using the anterior corpus callosum cluster as a seed region																		
Target regions																		
Anterior cingulate	1695	526	1719	671	0.03	>0.99	0.45	0.03	0.46	0.03	8.85	**0.03**	8.04	0.46	7.85	0.36	8.56	**0.04**
Middle frontal	2843	848	2863	852	0.66	>0.99	0.41	0.03	0.42	0.04	4.05	0.45	7.94	0.41	7.78	0.33	5.64	0.18
Frontal pole	2566	441	2580	504	0.09	>0.99	0.39	0.03	0.40	0.03	3.63	0.57	8.16	0.48	7.97	0.38	6.48	0.11
Superior frontal	3272	660	3276	655	1.02	>0.99	0.41	0.02	0.41	0.03	1.42	>0.99	8.11	0.45	7.94	0.36	4.16	0.42
Paracingulate	1214	638	1226	695	0.97	>0.99	0.47	0.03	0.47	0.03	1.68	>0.99	7.95	0.46	7.77	0.36	6.68	0.10
***A priori* tractography analyses of the uncinate fasciculus and cingulum**																		
Cingulum R	19284	15187	14637	11804	6.58	**0.04**	0.35	0.03	0.36	0.04	9.17	**0.01**	8.16	0.54	7.98	0.49	3.56	0.24
Cingulum L	19824	14641	17510	11854	1.02	>0.99	0.37	0.04	0.38	0.04	5.21	0.09	8.25	0.53	8.09	0.46	2.39	0.49
Uncinate R	22377	15118	20111	12774	0.02	>0.99	0.40	0.04	0.40	0.04	0.04	>0.99	7.85	0.40	7.77	0.31	0.86	>0.99
Uncinate L	17550	12637	15710	10673	0.02	>0.99	0.40	0.05	0.40	0.05	0.71	>0.99	8.01	0.47	7.88	0.35	0.09	>0.99

SD, standard deviation; F, ANOVA's F-value, p_c, p values corrected for multiple comparisons; **bold** figures indicate significant results at p<0.05
R, Right; L, Left.

Longitudinal analyses

TABLE S2. Association between neuroticism and hopelessness at age 14 with DTI measures

	Neuroticism			Hopelessness		
	β	t	p[†]	β	t	p[†]
mean FA values within the whole skeleton	-6.44×10^{-05}	-0.71	0.48	-2.91×10^{-05}	-0.13	0.87
mean FA values within the voxel-wise comparison cluster	-3.85×10^{-04}	-1.54	0.12	-4.61×10^{-04}	-0.75	0.46
mean FA values within the tracts spanning from voxel-wise comparison cluster to the ACC	-2.14×10^{-04}	-1.18	0.24	-4.38×10^{-04}	-0.98	0.33
mean FA values within the right cingulum bundle	-4.89×10^{-04}	-1.40	0.16	-6.00×10^{-04}	-0.69	0.49

[†] p values uncorrected for multiple comparisons; ACC: anterior cingulate cortex
Models were adjusted for age, sex, and DTI acquisition type.

Post-hoc analyses

Specificity of the findings in the study sample

The DAWBA "bands" distribution is described in ST3.

In the study sample, subthreshold-depression at age 14 was associated with higher risk at age 16 for generalized anxiety disorder (β=0.46, z=2.75, p=0.006), obsessive compulsive disorder (β=0.71, z=3.14, p=0.007), attention deficit hyperactivity disorder (β=0.84, z=3.89, p<0.001), and conduct disorders at follow-up (β=0.65, z=3.21, p=0.001). Subthreshold-depression did not predict social phobia (β=-0.01, z=-0.02, p=0.98), panic disorder (β=0.32, z=0.70, p=0.48), or oppositional-defiant disorder (β=0.19, z=1.71, p=0.09). Subthreshold-depression was also associated with higher risk for "any DSM-IV diagnosis" at age 16 (β=0.28, z=3.06, p=0.002).

In contrast, lower FA values did not predict higher risk for any other diagnosis than depression at follow-up (generalized anxiety disorder (β=-3.17, z=-1.13, p=0.26), obsessive compulsive disorder (β=-1.03, z=-0.29, p=0.78), attention deficit hyperactivity disorder (β=-3.04, z=-0.84, p=0.40), conduct disorders (β=-3.61, z=-1.03, p=0.30)). Lower FA values did not predict higher risk for "any DSM-IV diagnosis" (β=-1.03, z=-0.74, p=0.46) either.

TABLE S3. Distribution of DAWBA probabilities (bands) at age 16 in the study sample.

Diagnoses	Subthreshold depression participants DAWBA bands at age 16						Controls DAWBA bands at age 16					
	<0.1%	~0.5%	~3%	~15%	~50%	>70%	<0.1%	~0.5%	~3%	~15%	~50%	>70%
Depression	16	40	0	14	8	3	118	135	0	20	7	2
Generalized Anxiety Disorder	46	21	1	13	0	0	208	55	2	14	4	0
Social Phobia	16	58	3	4	0	0	51	218	4	6	5	0
Panic Disorder	75	4	2	0	0	0	274	5	3	1	1	0
Obsessive Compulsive Disorder	51	25	5	0	0	0	235	40	7	1	1	0
Attention Deficit Hyperactivity Disorder	55	16	3	3	2	0	235	24	12	4	0	0
Oppositional-Defiant Disorder	0	47	24	5	3	0	0	212	58	3	2	0
Conduct Disorder	57	16	2	4	1	1	237	35	1	6	0	4
Any DSM-IV diagnosis	_	19	32	18	8	3	_	146	90	31	12	5

Specificity of the clinical findings in the study sample

Causal mediation analyses showed that higher scores of neuroticism and hopelessness accounted respectively for 46% (p=0.02) and 41% (p=0.04) of the relationship between subthreshold-depression at baseline and depression at age 16 (ST4).

Additionally, higher scores of neuroticism and hopelessness were associated with higher risk for "any DSM-IV diagnosis" at age 16 ($\beta=0.021$, z=3.95, p<0.001 and $\beta=0.040$, z=3.17, p=0.002 respectively).

Causal mediation analyses showed that higher scores of neuroticism and hopelessness respectively accounted for 44% (p=0.01) and 25% (p=0.01) of the relationship between subthreshold-depression at baseline and the DAWBA probability of having "any DSM-IV diagnosis" at age 16 (ST5).

TABLE S4. Causal mediation analyses on the relationship between subthreshold-depression at age 14 and depression at age 16 with scores for neuroticism and hopelessness and as mediator.

Mediator variable: Score for neuroticism at age 14

Effect type	Point estimate	95% CI	p-value
Mediation effect (subthreshold-depression at 14 - neuroticism - depression at 16)	0.036	[0.010;0.073]	0.01
Direct effect (subthreshold-depression at 14 - depression at 16)	0.044	[-0.020; 0.131]	0.21
Total effect	0.080	[0.016; 0.172]	0.01
Proportion of total effect via mediation	0.464	[0.105; 1.864]	

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

Mediator variable: Score for hopelessness at age 14

Effect type	Point estimate	95% CI	p-value
Mediation effect (subthreshold-depression at 14 - hopelessness - depression at 16)	0.028	[0.010; 0.054]	0.04
Direct effect (subthreshold-depression at 14 - depression at 16)	0.039	[-0.025; 0.126]	0.25
Total effect	0.067	[0.004; 0.156]	0.04
Proportion of total effect via mediation	0.409	[0.081; 2.462]	

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

TABLE S5. Causal mediation analyses on the relationship between subthreshold-depression at age 14 and DAWBA probability for "any DSM-IV diagnosis" at age 16 with scores for neuroticism or hopelessness and as mediator.

Mediator variable: Score for neuroticism at age 14

Effect type	Point estimate	95% CI	p-value
Mediation effect			
(subthreshold-depression at 14 - neuroticism - probability of any diagnosis at 16)	0.246	[0.097;0.419]	0.01
Direct effect			
(subthreshold-depression at 14 - probability of any diagnosis at 16)	0.319	[-0.068; 0.732]	0.11
Total effect	0.565	[0.184; 0.970]	<0.001
Proportion of total effect via mediation	0.437	[0.158; 1.303]	

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

Mediator variable: Score for hopelessness at age 14

Effect type	Point estimate	95% CI	p-value
Mediation effect			
(subthreshold-depression at 14 - hopelessness – probability of any diagnosis at 16)	0.136	[0.032; 0.263]	0.01
Direct effect			
(subthreshold-depression at 14 - probability of any diagnosis at 16)	0.414	[0.049; 0.819]	0.03
Total effect	0.550	[0.192; 0.948]	0.01
Proportion of total effect via mediation	0.249	[0.055; 0.751]	

Point estimate: estimate of the size of the effect; 95% CI; 95% confidence interval of the point estimate.
Reproducibility of the findings in a distinct sample
In the 686 healthy adolescents at baseline, lower FA values in tracts spanning from the anterior corpus callosum cluster to the anterior cingulate cortex predicted higher risk for depression at age 16 (β=-0.04, z=-2.19, p=0.028).

Specificity of the findings in the distinct sample

The DAWBA "bands" distribution is described in ST6.

In the 686 healthy adolescents at baseline, lower FA values did not predict higher risk for any other diagnosis (generalized anxiety disorder (β=-0.01, z=-0.47, p=0.64), social phobia (β=-0.01, z=-0.43, p=0.66), panic disorder (β=-0.09, z=-1.32, p=0.19), obsessive compulsive disorder (β=-0.03, z=-0.76, p=0.45), attention deficit hyperactivity disorder (β=-0.05, z=-1.56, p=0.12), odd (β=-0.02, z=-1.05, p=0.29), or conduct disorders at follow-up (β=-0.01, z=-0.39, p=0.69). Lower FA did not predict risk for "any DSM-IV diagnosis" (β=-0.02, z=-1.18, p=0.24).

TABLE S6. Distribution of DAWBA probabilities (bands) at age 16 in 686 healthy adolescents at age 14.

	DAWBA bands at age 16					
Diagnoses	<0.1%	~0.5%	~3%	~15%	~50%	>70%
Depression	252	338	0	67	15	3
Generalized Anxiety Disorder	0	528	104	36	4	0
Social Phobia	218	412	12	22	10	0
Panic Disorder	645	21	0	6	2	0
Obsessive Compulsive Disorder	565	97	6	5	1	0
Attention Deficit Hyperactivity Disorder	544	96	23	1	0	0
Oppositional-Defiant Disorder	0	490	154	19	5	2
Conduct Disorder	532	100	12	24	15	3
Any DSM-IV diagnosis	_	293	247	108	40	4

REFERENCES

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