Appendix 1 to Bralten J, Greven CU, Franke B, et al. Voxel-based morphometry analysis reveals frontal brain differences in participants with ADHD and their unaffected siblings. *J Psychiatry Neurosci* 2015.

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Supplementary materials

Appendix A;

To determine ADHD diagnoses, a combination of Conners' ADHD questionnaires and a semi-structured diagnostic interview was used(1-4). Each participant was assessed with a parent-rated questionnaire (Conners' Parent Rating Scales – Revised: Long version [CPRS-R:L]) and either a teacher-rating (Conners' Teacher Rating Scales – Revised: Long version [CTRS-R:L] applied for children <18 years) or a self report (Conners' Adult ADHD Rating Scales–Self-Report: Long version [CAARS-S:L] applied for children ≥18 years). Participants were administered the Dutch translation of the Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version (K-SADS-PL: Kaufman et al., 1997) containing developmentally appropriate questions to assess each of the 18 ADHD symptoms, compatible with the DSM-IV-TR (American Psychiatric Association [APA], Diagnostic and Statistical Manual of mental disorders, Text Revision [DSM-IV-TR] 2000). Parents, reporting on their children, as well as children themselves, if ≥12 years old, were interviewed separately. Final scores on each item of the K-SADS were determined by weighing all available information. Initially, all participants were administered the K-SADS ADHD screening interview. Participants with elevated scores on any of the screen items were administered the full ADHD supplement. For participants using medication, ratings were gathered of participants' functioning off medication. Using a diagnostic algorithm, a combined symptom count was calculated by adding symptom counts on the K-SADS and CTRS-R:L (for participants <18) or CAARS-S:L (for participants≥ 18), both providing operational definitions of each of the 18 behavioural symptoms defined by the DSM-IV. Symptoms of the CTRS-R:L or CAARS-S:L were only added to the combined symptom count if at least 2 symptoms were reported, in order to avoid the Conners' score to put too much weight on the diagnosis.

From the Conners' ADHD questionnaires the following scales were used: DSM Inattentive behaviour (scale L of the CTRS-R:L/CPRS-R:L; scale E of the CAARS-S:L), DSM Hyperactive/Impulsive behaviour (scale M of the CTRS-R:L/CPRS-R:L; scale F of the CAARS-S:L), and DSM Total (scale N of the CTRS-

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R:L/CPRS-R:L; scale G of the CAARS-S:L). Participants with a combined symptom count of ≥6 symptoms of hyperactive/impulsive behaviour and/or inattentive behaviour were diagnosed with ADHD, provided they: a) met the DSM-IV criteria for pervasiveness and impact of the disorder (measures derived from the K-SADS), b) showed an age of onset before 12, c) received a *T* ≥63 on at least one of three scales on at least one of the Conners' ADHD questionnaires (i.e. CPRS-R:L and/or CTRS-R:L for children <18 years and CPRS-R:L and/or CAARS-S:L for children ≥18 years), pertaining to a period without medication.

Participants with a combined symptom count of ≥ 6 symptoms who did not meet one or more of these criteria were evaluated by a team of trained experts, in order to derive a consensus decision on their diagnosis. Unaffected participants were required to receive a *T*<63 on each of the above-mentioned scales of each of the Conners' ADHD questionnaires, and have a combined symptom count ≤3 symptoms. For young adults (≥ 18 years), criteria were slightly adapted, such that a combined symptom count of 5 instead of 6 symptoms was sufficient for a diagnosis (5) and with ≤2 symptoms on the combined symptom count they were labelled 'unaffected'. Diagnostic procedure for parents were similar to those applied for children ≥18 years. The ADHD questionnaire was completed by their partner (Conners' Adult ADHD Questionnaire-Observer: Short version [CAARS-O:SV]). A retrospective childhood diagnosis was established in addition to a current diagnosis, using the same diagnostic algorithm used for young adults. Parents with a current and/or childhood diagnosis were labelled as affected.

Appendix B;

MRI acquisition and preprocessing: MRI was conducted at two different locations (Donders Centre for Cognitive Neuroimaging in Nijmegen and VU University Medical Centre in Amsterdam), using two comparable 1.5 Tesla MRI scanners (Sonata/Avanto Siemens, Munich, Germany), the same 8-channel head-coil and scan protocols. For each participant we obtained two high-resolution T1-weighted MPRAGE anatomical scans (176 sagittal slices, TR = 2730

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msec, TE = 2.95 msec, TI=1000 ms, flip angle = 7 deg, GRAPPA 2, voxel size = 1.0 x 1.0 x 1.0 mm, field of view = 256 mm), one before and one after a break in a longer scanning session. MRI scans which yielded clinically relevant incidental findings (n=16), and those for which manual ratings revealed poor quality or motion artifacts (n=4, all belonging to the ADHD group) were excluded from analysis(6). For participants who had two good scans, we averaged the VBM estimates across both scans, thereby improving signal-to-noise. If only one good scan was available, a single scan was used. Each participant's T1-weigthed scan was normalized to Montreal Neurological Institute (MNI) 152 standard space, bias-field corrected and segmented into grey matter, white matter, and cerebrospinal fluid using the unified procedure of the VBM 8.1 toolbox (http://dbm.neuro.uni-jena.de/vbm/) in SPM (default settings)(7). This method uses an optimized VBM protocol(8, 9) as well as a model based on Hidden Markov Random Fields (HMRF) developed to increase signal-to-noise ratio(10). Correction for total brain volume was incorporated in the toolbox. All data were smoothed with an 8 mm FWHM Gaussian smoothing kernel. Data analysis was restricted to voxels with grey matter with a probability exceeding 25% leading to inclusion of a total of 230,135 voxels.

Appendix C;

The main analysis was rerun using SPM defaults to compare our results to a standard VBM procedure not correcting for the family dependence in our data. As expected, inclusion of siblings within the group analysis increased both the within group similarity and between group differences, making the clusters more defined (smaller) when correcting for family dependence. The results without this correction (see Supplementary figure S1 and supplementary table S1) are within the same areas, but larger. While 5 distinct clusters are reported in the main analysis, they cluster into one large cluster in this analysis. Also a cluster in the precuneus reaches significance which is subthreshold in our main analysis.

Appendix D;

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A series of additional sensitivity analyses were conducted to examine possible effects of the factors of gender, IQ and scan-site within our sample. To ensure that none of the reported effects in the main body of the manuscript were dependant on differences between the groups in these factors, the main analyses were repeated with matched groups on each of these factors. Since this matching lead to the exclusion of a significant part of our sample, in particular the most severe patients with ADHD, we present these data additionally with the side note that we have reduced power and are looking at a more atypical ADHD group.

The distribution of gender, IQ, and scan-site distribution differed between patients and healthy controls in this study. In addition to covarying gender, IQ and scan-site in the main analyses, we repeated the main analyses of the diagnostic group contrast of interest in subgroups of our sample matched on gender, IQ and scan-site distribution respectively. Gender matched groups were achieved by equalizing the ratio of females in the control group to the ADHD group. This lead to the exclusion of 55 females from the controls. IQ matched groups were achieved by subdividing the IQ scores into seven bins of 15 points each. Subsequently, the ratio of patients and controls was equalized to the mean of the entire sample for each bin. This led to the exclusion of 71 patients from the lower three bins, as well as 45 controls from the upper four. For matching the scan-site distribution we equalized the ratio of females and males in both the control group and the ADHD group at both sites. This lead to the exclusion of 29 male controls at site 1 and 47 female ADHD participants at site 2.

Comparison of the results of the analyses on the matched subgroups can be found in supplementary table S8. These results showed that the direction of the neural effects is the same for all peak voxels in all sub-analyses. Due to a reduction of the sample size, not all clusters remained whole-brain significant. However, all sub-analyses showed whole-brain significant clusters, despite the reduction in power. In the gender-matched sub-analysis 3 clusters remained whole-brain significant (overlap with cluster 2 (orbitofrontal), cluster 3 (frontal pole) and cluster 4 (frontal pole) of the main analysis). In the IQ-

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matched sub-analysis 3 clusters remained whole-brain significant (overlap with cluster 1 (precentral), cluster 2 (orbitofrontal) and cluster 5 ((para)cingulate) of the main analysis). In the scan-site matched subsample 2 clusters remained whole-brain significant (overlap with cluster 2 (orbitofrontal) and cluster 3 (frontal pole) of the main analysis).

Supplementary table S1. Whole brain VBM differences between ADHD cases and controls using default SPM settings

	Size (nr voxels)	MNI coordinates (x,y,z)	Best z-value	Side of the brain	Area
Cluster 1	11745	-15,53,12	-5.23	Left	Paracingulate, superior frontal gyrus, frontal pole
Cluster 2	2850	-2,72,38	-4.16	Left	Precuneus cortex, cuneal cortex

Note Reported areas are identified with the Harvard Oxford cortical and subcortical structural atlases. Both clusters show smaller volume in subjects with ADHD compared to controls. MNI coordinates are provided in mm for the peak voxel. Cluster 1 corresponds with the 5 clusters reported in the main analysis. Cluster 2 was subthreshold of the accepted significance level in the main analysis.

Supplementary table S2. Medication use

	Mean voxel values of ADHD-medicated individuals (n=272)	Mean voxel values of ADHD-never medicated individuals (n=35)	p-value

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Cluster 1	0.4673 (0.004077)	0.4582 (0.0087274)	0.3419
Cluster 2	0.6267 (0.0043744)	0.6250011 (0.0138228)	0.9024
Cluster 3	0.3838 (0.0035979)	0.3966394 (0.009359)	0.1970
Cluster 4	04746734. (0.0036368)	0.4819023 (0.0082561)	0.4172
Cluster 5	0.6328908 (0.0047409)	0.62091 (0.0122998)	0.3575

Note Marginal mean voxel values and standard errors of the identified clusters for ADHD samples that ever used medication as well as never used medication are shown in the table. Corresponding uncorrected p-values for the difference between the two groups are provided. Medication use was unknown for 26 ADHD samples, which were excluded from this analysis.

Supplementary table S3. Age*diagnosis and age^2*diagnosis interactions

	Age*diagnosis	Age^2*diagnosis
Cluster 1	0.89	0.15
Cluster 2	0.78	0.70
Cluster 3	0.81	0.79
Cluster 4	0.88	0.96
Cluster 5	0.09	0.10

Note uncorrected p-values for the age*diagnosis and age^2*diagnosis interactions for the identified clusters.

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Supplementary table S4. Gender*diagnosis interactions

	Gender*diagnosis
Cluster 1	0.08
Cluster 2	0.14
Cluster 3	0.64
Cluster 4	0.99
Cluster 5	0.53

Note uncorrected p-values for the gender*diagnosis interactions for the identified clusters.

Supplementary table S5. Inclusion of IQ as a covariate

	Size (nr voxels)	MNI coordinates (x,y,z)	Best z-value	Side of the brain	Area
Cluster 1	124	-12,50,20	-3.84	Left	Paracingulate, cingulate, frontal pole
Cluster 2	164	28,70,-2	-3.99	Left	Frontal pole

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Cluster 3	171	-18,-72,38	-3.65	Right	Cuneal cortex
Cluster 4	214	-26,16,-24	-4.32	Left	Frontal orbital cortex
Cluster 5	616	2,20,-2	-3.82	Left and right	Frontal medial cortex, paracingulate, cingulated, subcallosal cortex

Note Identified whole-brain significant clusters when IQ was included as a covariate. MNI coordinates are provided in mm for the peak voxel.

Supplementary table S6. Smoking status

	Never smoked	Ever smoked	Unknown
ADHD	130	163	14
Unaffected Siblings	105	60	4
Controls	119	69	8

Supplementary table S7. Comparison scannersites for main findings of the ADHD control comparison.

	MNI coordinates (x,y,z)	Best z-value main analysis	Best z-value site 1	Best z-value site 2
Cluster 1	-40,-6,56	-3.96	-3.49	-2.56

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Cluster 2	-26,16,-24	-4.43	-3.38	-3.16
Cluster 3	28,70,-2	-4.17	-2.84	-3.53
Cluster 4	-14,52,14	-4.43	-5.22	-1.82
Cluster 5	2,22,-2	-3.79	-3.05	-2.48

Note Best z-values for the peak voxels from the main analysis, the subanalysis in site 1 and the subanalysis in site 2. MNI coordinates are provided in mm for the peak voxel.

Supplementary table S8. Peak voxel values main findings of the ADHD control comparison in the subsample analyses matched on gender, IQ and scan-site.

	MNI coordinates (x,y,z)	Best z-value main analysis	Best z-value gender-matched sample	Best z-value IQ-matched sample	Best z-value scan-site-matched sample
Cluster 1	-40,-6,56	-3.96	-3.39	**-4.33**	-3.21
Cluster 2	-26,16,-24	-4.43	**-4.44**	**-3.84**	**-4.34**
Cluster 3	28,70,-2	-4.17	**-4.44**	-3.53	**-3.75**
Cluster 4	-14,52,14	-4.43	**-3.94**	-3.11	-3.14
Cluster 5	2,22,-2	-3.79	-2.89	**-3.80**	-2.67

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Note Best z-values for the peak voxels from the main analysis, the analysis in a subsample matched on gender, the analysis in a subsample matched on IQ and the analysis in a subsample matched on scan-site. Z-values are indicated in bold if (part of) the cluster remained whole-brain significant in the subsample analyses. MNI coordinates are provided in mm for the peak voxel.

Supplementary table S9. Symptom count analyses.

	Size (nr voxels)	MNI coordinates (x,y,z)	Best z-value	Side of the brain	Area	Overlap ADHD-control analyses
Cluster 1	138	-28,-84,-18	-3.97	Right	Occipital fusiform gyrus	No
Cluster 2	141	-26,14,-28	-4.01	Left	Frontal orbital cortex	Yes
Cluster 3	143	-10,-44,54	-3.90	Right	Precuneous	No
Cluster 4	164	36,24,12	-3.95	Left	Inferior frontal gyrus, frontal operculum	No
Cluster 5	222	20,70,10	-3.84	Left	Frontal pole	Yes
Cluster 6	250	-28,58,18	-3.74	Left	Frontal pole	Yes
Cluster 7	1484	0,32,-6	-4.06	Left	Frontal medical cortex, paracingulate, cingulate,	Yes

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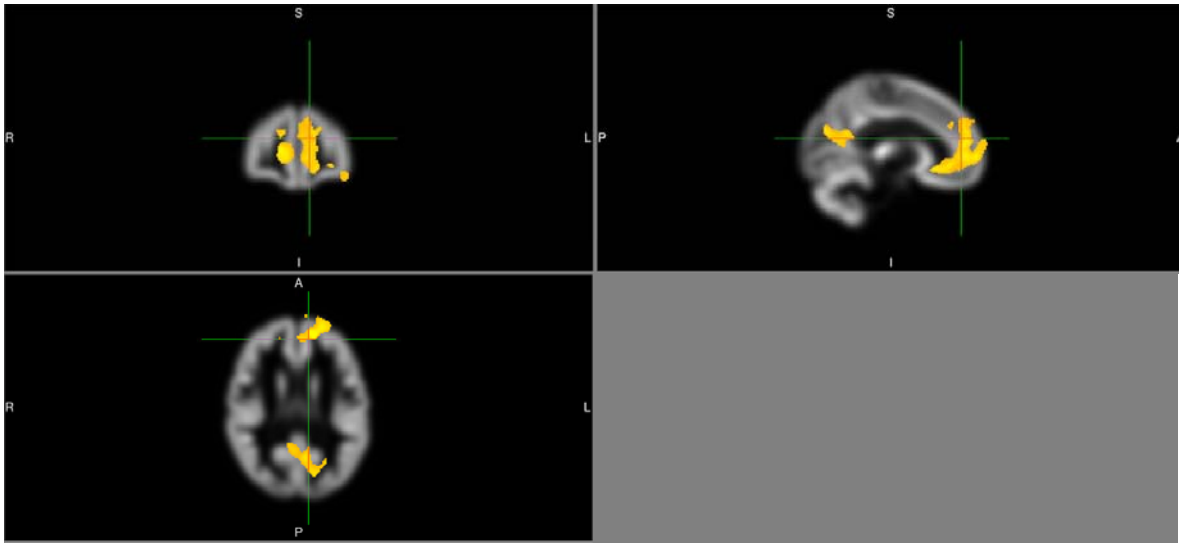
Note Identified whole-brain significant clusters for ADHD symptoms. MNI coordinates are provided in mm for the peak voxel.



Supplementary figure S1. Whole-brain significant clusters for case-control differences when family dependence was not taken into account. The 5 clusters reported in our main analysis cluster into one blob in the analysis not taking family dependence into account. The second cluster in the precuneus was subthreshold of the accepted significance level in the main analysis.

Supplementary figure S2. Different orientations of our main clusters. A: Anterior, P: Posterior, S: Superior, I: Inferior, R: Right, L: Left.

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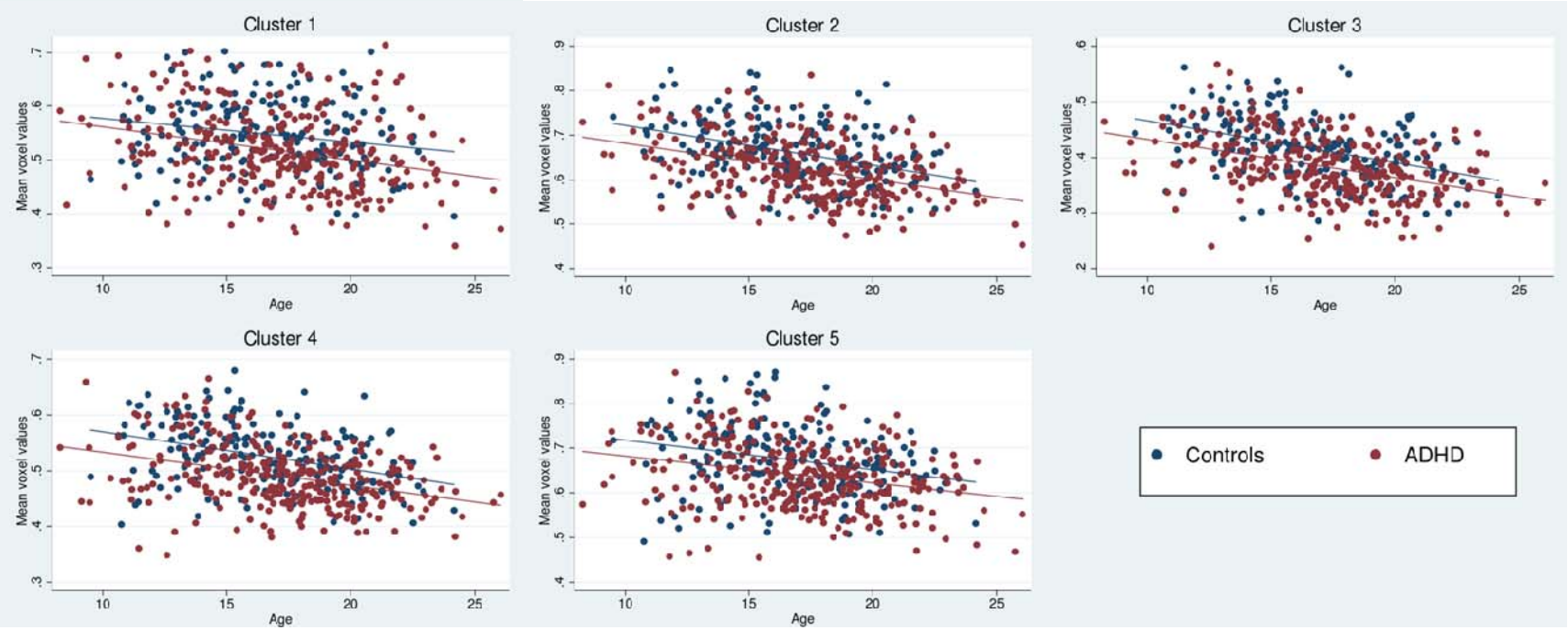
[Figure S2 here]



Supplementary Figure S3. For exploratory purposes we plotted the mean voxel values for each cluster per group by age. No age * diagnose interaction was significant.

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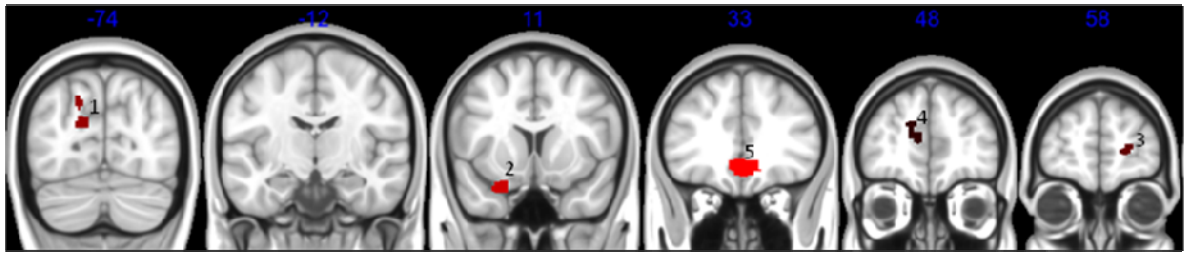

Supplementary Figure S4. Whole-brain significant clusters for case-control differences when IQ was included as a covariate. Cluster 1 in the cuneal cortex was not identified in the analysis without IQ as a covariate. Cluster 2,3,4 and 5 are identified in both analyses (with an without inclusion of IQ).

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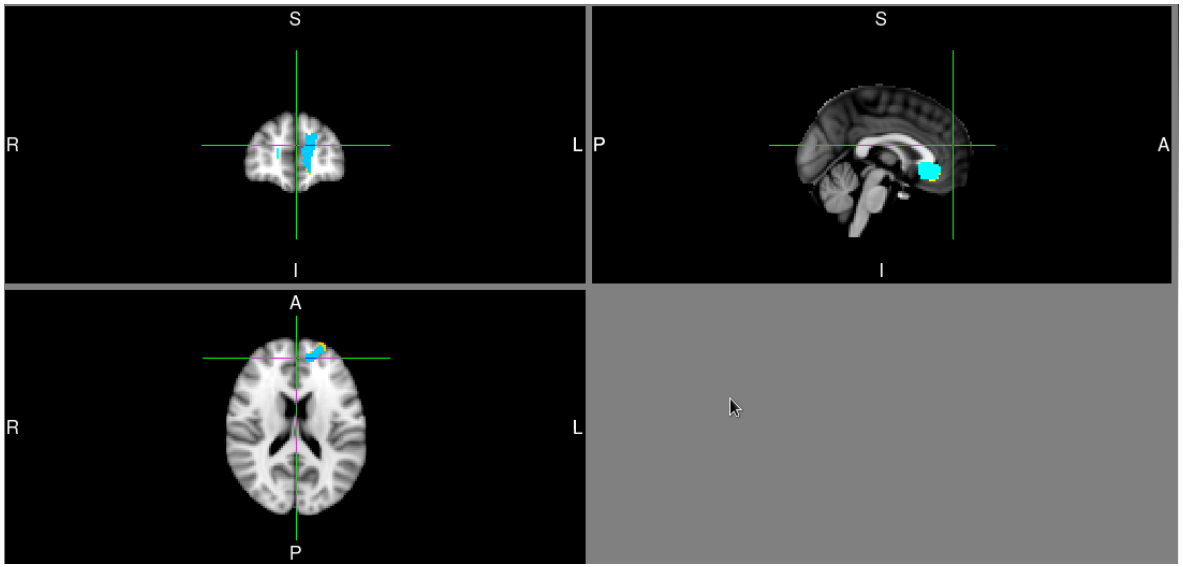

Supplementary Figure S5. Whole-brain significant clusters for case-control differences when smoking was included as a covariate are shown in blue. All five clustered from the main analysis were found back and overlap (main analyses clusters shown in yellow). A: Anterior, P: Posterior, S: Superior, I: Inferior, R: Right, L: Left.

1. Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, et al. Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. Journal of the American Academy of Child and Adolescent Psychiatry. 1997;36(7):980-8.

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2. Conners C, Sitarenios G, Parker J, Epstein J. Revision and restandardization of the Conners Teacher Rating Scale (CTRS-R): factor structure, reliability, and criterion validity. J Abnorm Child Psychol. 1998;26(4):279-91.

3. Conners C, Sitarenios G, Parker J, Epstein J. The revised Conners' Parent Rating Scale (CPRS-R): factor structure, reliability, and criterion validity. J Abnorm Child Psychol. 1998;26(4):257-68.

4. Conners CK, Erhardt D, Sparrow EP. Conner's Adult ADHD Rating Scales: CAARS: Multi-Health Systems, North Tonawanda, NY; 1999.

5. Kooij JJ, Buitelaar JK, van den Oord EJ, Furer JW, Rijnders CA, Hodiamont PP. Internal and external validity of attention-deficit hyperactivity disorder in a population-based sample of adults. Psychol Med. 2005;35(6):817-27.

6. Blumenthal JD, Zijdenbos A, Molloy E, Giedd JN. Motion artifact in magnetic resonance imaging: implications for automated analysis. NeuroImage. 2002;16(1):89-92.

7. Ashburner J. A fast diffeomorphic image registration algorithm. NeuroImage. 2007;38(1):95-113.

8. Ashburner J, Friston KJ. Voxel-based morphometry - The methods. NeuroImage. 2000;11(6):805-21.

9. Good CD, Johnsrude IS, Ashburner J, Henson RNA, Friston KJ, Frackowiak RSJ. A voxel-based morphometric study of ageing in 465 normal adult human brains. NeuroImage. 2001;14(1):21-36.

10. Cuadra MB, Cammoun L, Butz T, Cuisenaire O, Thiran JP. Comparison and validation of tissue modelization and statistical classification methods in T1-weighted MR brain images. IEEE transactions on medical imaging. 2005;24(12):1548-65.

Appendix 1 to Bralten J, Greven CU, Franke B, et al. Voxel-based morphometry analysis reveals frontal brain differences in participants with ADHD and their unaffected siblings. *J Psychiatry Neurosci* 2015.

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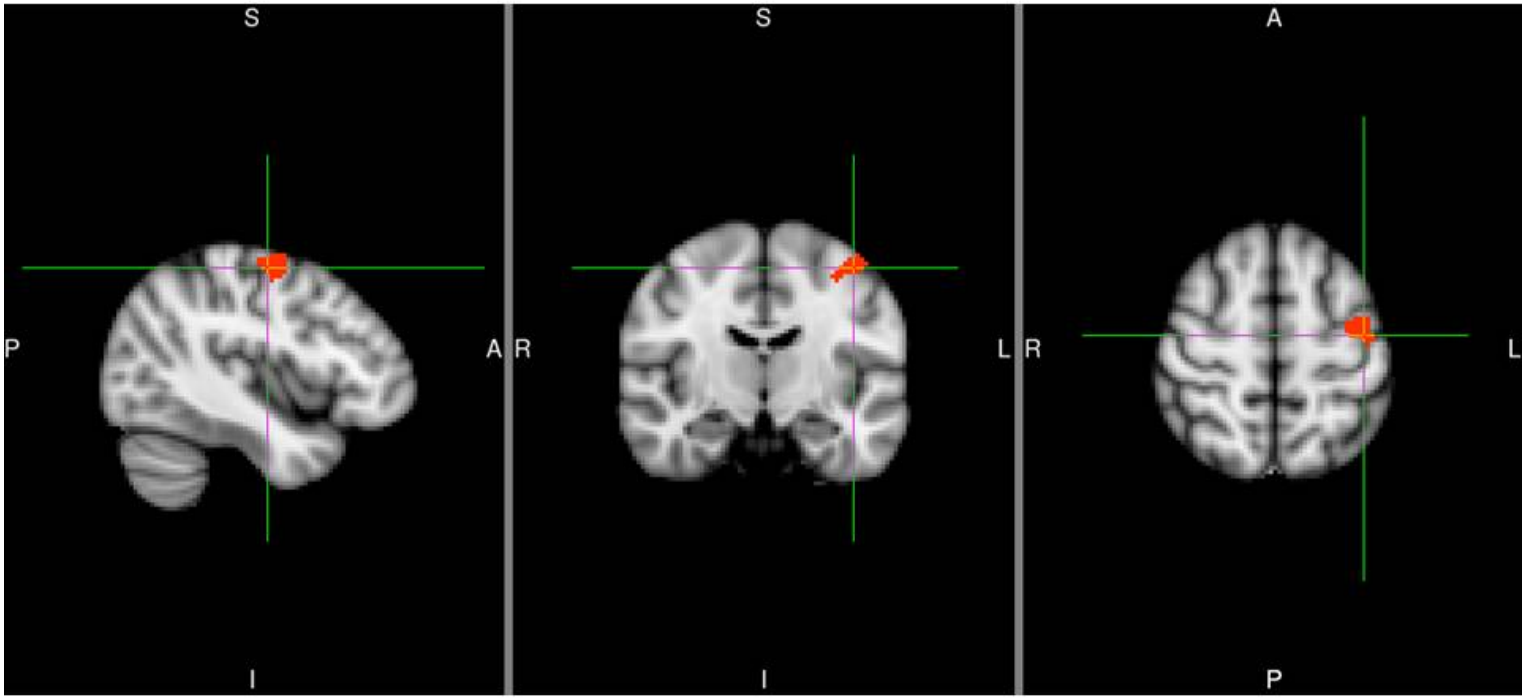
Cluster 1 (-42,-12,56)



A: Anterior, P: Posterior, S: Superior, I: Inferior, R: Right, L: Left

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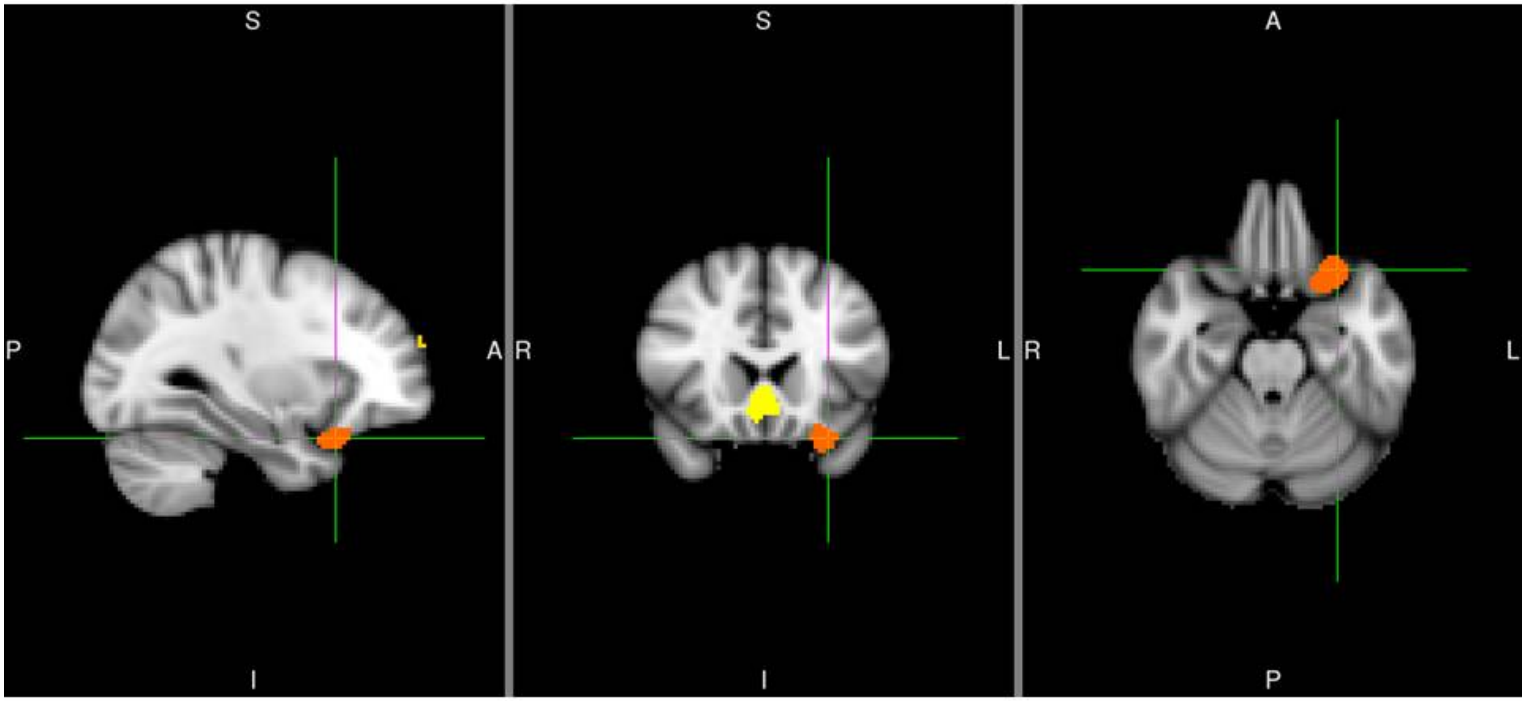
Cluster 2 (-30,20,-24)



A: Anterior, P: Posterior, S: Superior, I: Inferior, R: Right, L: Left

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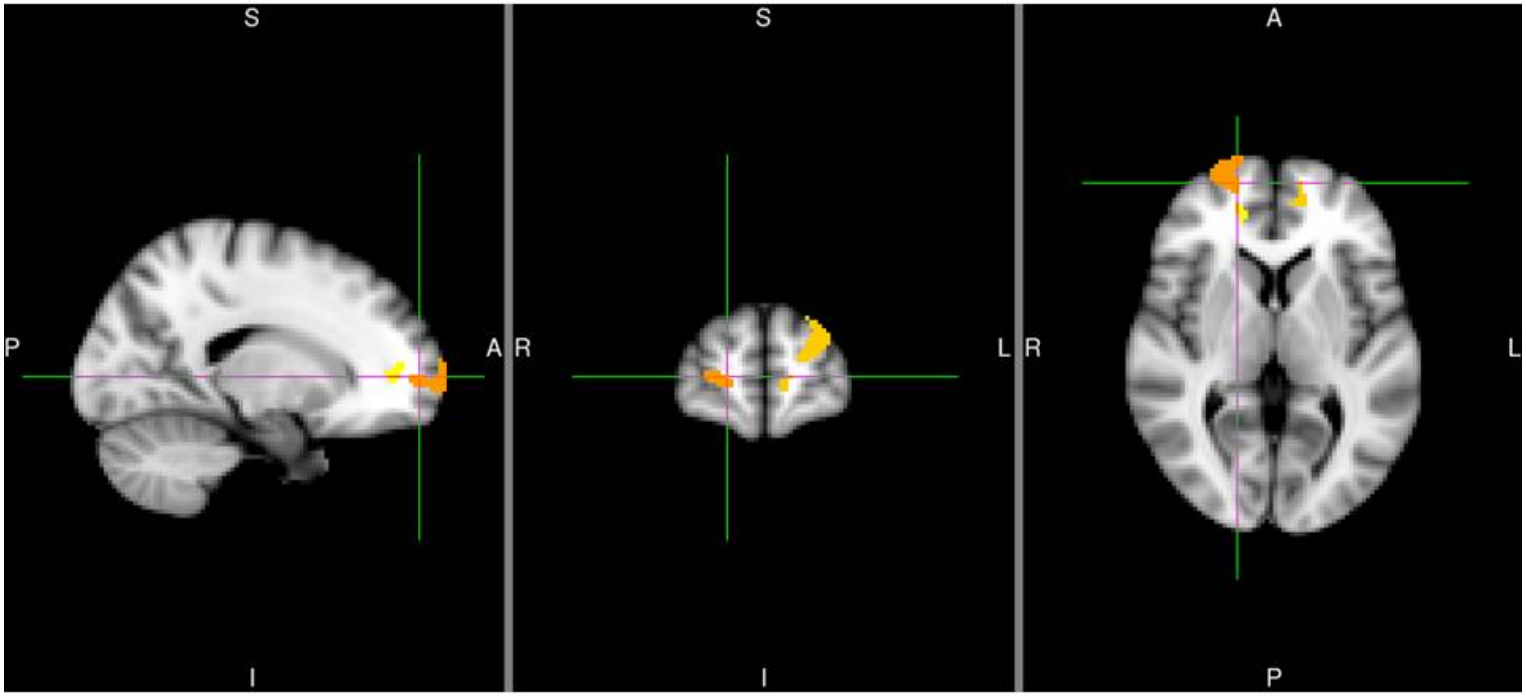
Cluster 3 (18,60,4)



A: Anterior, P: Posterior, S: Superior, I: Inferior, R: Right, L: Left

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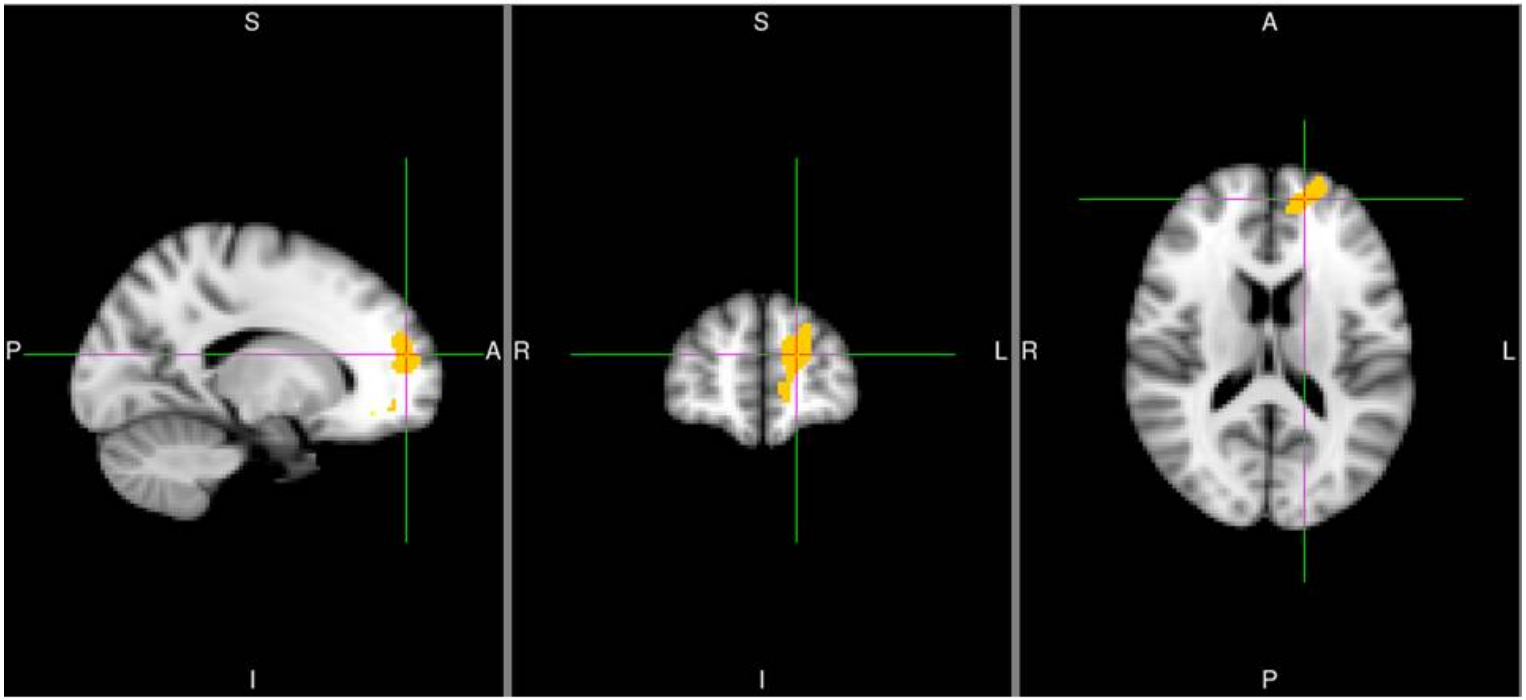
Cluster 4 (-16,54,16)



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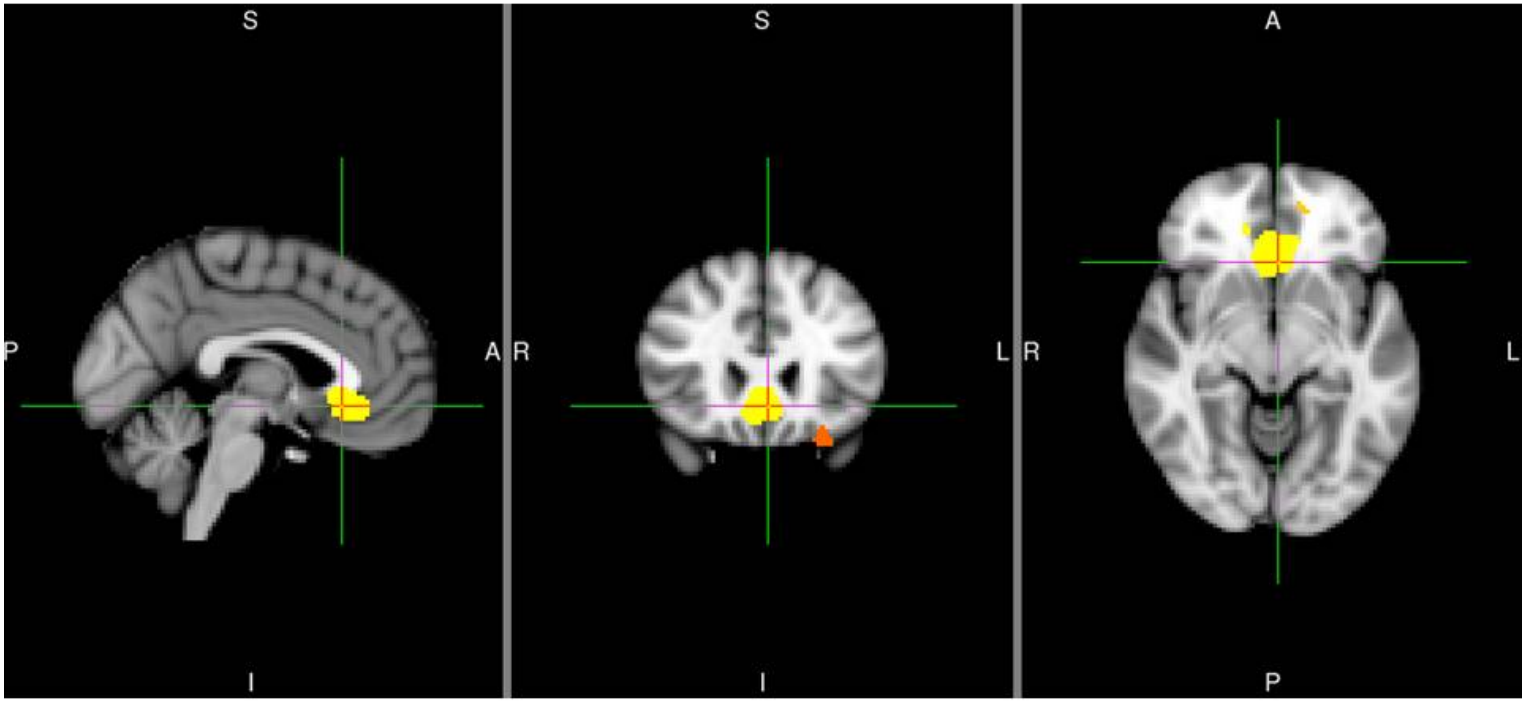
Cluster 5 (-2,24,-8)



A: Anterior, P: Posterior, S: Superior, I: Inferior, R: Right, L: Left