MS50–Report on the longitudinal MRI analyses of compulsivity in the NeuroIMAGE cohort

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1. Executive Summary

Low volume in clusters located in prefrontal, frontal, precentral and cerebellar regions were associated with persistent versus remittent ADHD and/or smaller ADHD symptom decline over time. Conversely, low volume in visual/auditory cortices was related to ADHD remission. ADHD full-persisters had significantly larger left amygdala volumes relative to controls. Significantly higher polygenic risk scores (PRS) for ADHD were seen in both persisters and remitters relative to controls. However, no significant associations between ADHD-PRS and ADHD persistence/remission were observed in either the categorical or dimensional analyses.

The analysis of white matter infrastructure showed that improvement in hyperactive/impulsive symptoms was associated with lower FA and higher MD values in the left corticospinal tract at follow-up. However, changes in white matter microstructure did not significantly differ between participants with and without ADHD. Furthermore, change in ADHD score was not significantly related to the change in white matter microstructure as measured using FA and MD indices.

Higher connectivity within frontal regions (anterior cingulate cortex) of the executive control network was related to decreases in ADHD symptoms. This association was driven by change in hyperactive/impulsive symptoms and not by change in inattention. Participants with remitting ADHD showed stronger RSFC than controls within this network, while persistent ADHD cases exhibited RSFC strengths intermediate to remittent ADHD cases and controls. Cerebellar and subcortical RSFC did not differ between participants with ADHD and controls.

2. Milestone report

See detailed info in the following (submitted) manuscripts:

- Adamo, N. et al. (2018) “Testing the differences in polygenic risk scores for ADHD between ADHD persistence and remission in a follow up of two European clinical samples” (submitted)
- Francx et al. (2015). Development of ADHD symptoms and white matter microstructure (internal report)

3. Tables and other supporting documents where applicable and necessary

See the manuscripts

4. Acknowledgement and Disclaimer

The research leading to these results has received funding from the European Community’s Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 278948.

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Grey matter volume relates to persistence and remission of ADHD symptoms and diagnosis

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Grey matter volume relates to persistence and remission of ADHD symptoms and diagnosis

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Grey matter volumes in ADHD persistence/remission

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Conflict of Interest Disclosures

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advisory board of and/or speaker for Janssen Cilag BV, Eli Lilly, Bristol-Myer Squibb, Shering Plough, UCB, Shire, Novartis and Servier; he is not an employee of any of these companies, nor a stock shareholder of any of these companies; he has no other financial or material support, including expert testimony, patents, and royalties. J Kuntsi has given talks at educational events sponsored by Medice; all funds are received by King’s College London and used for studies of ADHD. No other disclosures were reported.
ABSTRACT

Objective: Clinical outcome for childhood-onset attention-deficit/hyperactivity disorder (ADHD) in late adolescence and early adulthood ranges from full persistence to full remission of symptoms and functional impairment. Few studies have examined whether and which structural brain measures are associated with persistence/remission of ADHD.

Method: Participants were 238 youths with combined-type ADHD and 147 typically-developing controls from a 6-year follow-up study (mean age at follow-up: 17.5 years). At follow-up, participants with ADHD were classified as having full-persistent combined-type ADHD (n=100), partial-persistent ADHD (i.e., Inattentive or Hyperactive/Impulsive type, n=106) or remitted ADHD (n=32). Cross-sectional analyses of total brain volume, subcortical volumes and voxel-based morphometry (VBM) obtained at follow-up tested whether brain measures differentiated groups based on current diagnostic status, and whether brain measures were associated with a change in ADHD symptoms from baseline to follow-up.

Results: VBM analysis showed that low volume in clusters located in prefrontal, frontal, precentral and cerebellar regions were associated with full-/partial-persistent ADHD and/or smaller ADHD symptom decline over time. Conversely, low volume in visual/auditory cortices was related to ADHD remission. ADHD full-persisters had significantly larger left amygdala volumes relative to controls.

Conclusions: Low grey matter volumes in regions involved in top-down cognitive control (frontal/cerebellar cortices) appear to contribute to the persistence of ADHD. Findings suggest possible earlier maturation in visual/auditory cortices in remitters, supporting previous theories that over-reliance on sensory processing might compensate for frontal control deficits in ADHD. Future studies should investigate possible links of amygdala structural alterations with emotional regulation problems in ADHD persistence.
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INTRODUCTION

Clinical outcomes for children with attention-deficit/hyperactivity disorder (ADHD) into adolescence and adulthood range from full remission of symptoms and functional impairment, to improvement in one symptom domain (inattention or hyperactivity-impulsivity), to full persistence of an ADHD diagnosis (1-4). Compared to controls, individuals with ADHD persistence into adulthood show worse educational, economic and social outcomes, higher death rates, and risk for psychiatric hospitalizations and incarcerations (1, 5). Although various anatomical and functional brain abnormalities are reported in individuals with ADHD across the life-span and most prominently in childhood (6, 7), the neural mechanisms that contribute to persistence or remission of ADHD are largely unknown. Measures of brain structure have gained substantial interest as potential markers of typical and atypical brain development (8, 9).

Individuals with a current diagnosis of ADHD have a 3-5% smaller total brain size compared to controls (10-14), which was confirmed in a mega-analysis of the largest aggregate dataset of brain scans in individuals with ADHD to date (7). Evidence is also accumulating of lower volumes in frontal areas, basal ganglia, amygdala, hippocampus and cerebellum in individuals with ADHD compared to controls (7, 11, 14, 15). Using a voxel-based morphometry (VBM) approach, smaller volumes emerged in four clusters in the medial, orbital and lateral frontal cortices in a uniquely large sample of individuals with ADHD compared to controls (16). Such volumetric differences are in line with evidence from structural and functional studies that ADHD is associated with abnormalities in fronto-subcortical, fronto-parieto-temporal, fronto-cerebellar and fronto-limbic networks, which are putatively implicated in cognitive, visual-motor and emotional control impairments in ADHD (6, 7, 17).
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Delayed maturation of brain structure has been hypothesized in individuals with ADHD (12, 18). Cortical thinning prominently located in medial, superior prefrontal and precentral areas is associated with a greater number of persistent ADHD symptoms in adulthood, while individuals with ADHD remission show similar cerebral cortical structure to neurotypical individuals (19, 20). Normalization in these regions in ADHD remission corroborates an existing developmental model proposing that higher-level cognitive functions, typically linked to the functioning of the prefrontal cortex, improve with ADHD remission (21). Yet, most studies to date have not found alterations in cognitive and brain measures of prefrontal functions to be related to ADHD persistence and remission (22-25). Therefore, non-frontal and/or subcortical brain networks may also play a role in the persistence/remission of the disorder.

Investigations of the role of grey matter volume in persistence/remission of ADHD are so far limited. One recent study identified reduced volume in the caudate nucleus in adults with persistent ADHD, but no remitted ADHD cases were included for comparison (24). So far, only one study has investigated brain volumes in ADHD outcomes by following children with ADHD from childhood to adulthood (26). Using VBM in a cross-sectional study design, no difference was found in grey matter volume between ADHD persisters and remitters at follow-up (26). Instead, smaller cerebellar and subcortical volumes (i.e., thalamus and caudate nucleus) emerged, relative to controls, in all individuals with a history of childhood ADHD, independent of the current diagnostic status (26). No study to date has considered total grey-matter volumes in the persistence and remission of ADHD, and the results summarized above are based on relatively small sample sizes and await replication.

Previous work has shown considerable individual variation in developmental trajectories of ADHD symptoms. In a clinical follow-up of youths with ADHD, the persistence of the combined clinical
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presentation of inattentive plus hyperactive-impulsive symptoms appeared to be the most frequent (27). However, studies revealed that, through development, a high number of children show remission in at least one ADHD subdomain and shift from the combined to the predominantly inattentive presentation (27, 28). Thus categorical definitions of persistence/remission may be somewhat arbitrary and limited. Categorical analyses that take into account also partial remission and dimensional approaches may help understand how structural brain patterns reflect changes in the clinical presentation of ADHD over time.

Using whole-brain and subcortical volumes as well as VBM measures, this study aims to identify structural brain characteristics that associate with the persistence/remission of ADHD in a clinical 6-year follow-up of participants with ascertained DSM-IV ADHD Combined Subtype in childhood. To do so, we build on previous analyses in this sample (10, 16) which demonstrated total brain and localized grey matter volume reductions in participants with a current ADHD diagnosis. Specifically, we extend this investigation to ADHD remitters – those without a diagnosis at follow-up – and by examining the association of brain measures with the persistence/remission of the ADHD diagnosis and changes in the number of ADHD symptoms from childhood-adolescence to follow-up. We first investigate whether reduced global and localized brain volumes distinguish full syndromic persistence from partial and full ADHD remission as well as from non-ADHD comparisons. Second, we examine the association of brain measures with the change in ADHD symptoms over time in individuals with childhood ADHD.
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METHODS

Participants

Participants were recruited from the NeuroIMAGE project, a follow-up of the Dutch part of the International Multicentre ADHD Genetics (IMAGE) study (29). The IMAGE study recruited ADHD families with one child with combined-type ADHD and at least one biological sibling (regardless of ADHD diagnosis), as well as control families with at least one child and one biological sibling with no formal or suspected ADHD diagnosis in first-degree family members. Inclusion criteria for the IMAGE study were an age of 5-19 years, Caucasian descent, IQ≥70, no diagnosis of autism, epilepsy, general learning difficulties, brain disorders and known genetic disorders. Sampling methods and study procedures of the NeuroIMAGE project have been detailed previously (30). Inclusion criteria were largely consistent with the IMAGE Study, except that inclusion of any ADHD subtype was allowed. The study was approved by the Dutch local medical ethics committees. Written informed consent was obtained for all participants.

Following exclusion for contraindications to MRI scanning and quality control of MRI scans (described further on), we included 394 individuals who took part in the follow-up study (mean [SD] follow-up time: 6.7 [0.77] years): 247 had a diagnosis of DSM-IV combined-type ADHD (174 singletons and 74 siblings) and 147 were individuals from control families (33 singletons and 114 siblings) at initial assessments. At follow-up, participants with ADHD were classified as having full-persistent ADHD (i.e., combined type, n=100), partial-persistent ADHD (i.e., inattentive or hyperactive-impulsive type, n=106) or remitted ADHD (n=32). For details on diagnostic assessments, see the online supplement. Group characteristics are shown in Table 1.
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MRI data acquisition

The MRI scanning was conducted at follow-up at two different locations in the Netherlands using comparable 1.5-T MRI scanners (Siemens Sonata/Avanto) and the same 8-channel head-coil and scan protocols. For each participant, we obtained two high-resolution T1-weighted magnetization-prepared rapid acquisition with gradient echo anatomic scans: one before and one after a break in a longer scanning session. For participants with two good scans, we averaged the volume and VBM estimates across both scans, thereby improving the signal-to-noise ratio. If only one good scan was available, we used a single scan.

Volume estimation

Normalization, bias correction, and segmentation into gray matter, white matter, and cerebrospinal fluid volumes were performed using the unified procedure of the SPM-VBM 8.1 tool-box (http://dbm.neuro.uni-jena.de/vbm/; default settings).

Whole-brain and subcortical volumes: Total gray and white matter volumes were calculated by summation of their tissue probability maps. Total brain volume was the sum of total gray and white matter volumes. Following the same procedures applied in (10), automated FIRST (FMRIB’s Integrated Registration and Segmentation Tool) subcortical segmentation was applied to estimate total, left and right volumes of the amygdala, caudate nucleus, hippocampus, nucleus accumbens, globus pallidus, putamen, thalamus, and brainstem.

VBM: To obtain VBM measures, we followed the same procedures implemented in (16). Grey matter images were modulated by the nonlinear part of the normalization field and smoothed with an 8-
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mm full-width at half-maximum Gaussian kernel, allowing for analysis of relative differences in regional grey matter volume, corrected for individual brain size (http://dbm.neuro.uni-jena.de/vbm8/VBM8-Manual.pdf). Data analysis was restricted to voxels with a grey matter probability exceeding 25%, leading to inclusion of 583,291 voxels.

**Statistical analysis**

Statistical analyses were conducted using Stata (StataCorp LP). Preliminary regression analyses tested whether the childhood-ADHD and control groups showed the global and localized brain volumetric differences reported previously in a partially overlapping sample (10, 16). The model included binary diagnosis (ADHD and control) as a main effect and whole-brain volumes, subcortical volumes and the voxel-wise grey matter volume value at each voxel as outcome measures. Then, following the study aims, we tested the difference in brain measures between ADHD full-persisters, partial-persisters, remitters and controls, by regressing each brain volume measure on current diagnostic status (full-persister, partial-persister, remitter, control) as the group factor. Finally, among participants with childhood ADHD, we tested whether brain volumes were associated with the ADHD symptom change using linear regression analyses that modelled each brain volume value as a function of the participant’s change in ADHD symptom count (difference between the total ADHD symptom count at baseline minus the total symptom count at follow-up).

Outlier total brain and subcortical volumes exceeding 3 SDs from the sample mean were removed. Sex, age at follow-up, age² and scanner location were entered in all models as covariates of no interest. Total brain volume was included as an additional covariate for regressions of subcortical volumes to enable inferences about subcortical alterations unconfounded by total brain volume. As observations were not independent within families, we used the “robust cluster” command in Stata,
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which accounts for the correlation structure of the data in calculating robust standard errors (31).

On measures that yielded a significant association with diagnostic group or ADHD symptom change, post-hoc regressions were performed to check the impact of age, sex, IQ and lifetime ADHD medication use on the results. Information on lifetime use of ADHD medications (atomoxetine, dexamphetamine, immediate-release and extended-release methylphenidate) was gathered from pharmacy transcripts and questionnaires.

To correct for multiple testing in the neuroanatomical volumes analysis, we estimated the equivalent number of effective variables using the correlation matrix of the included brain measures (http://gump.qimr.edu.au/general/daleN/matSpD/), and performed Bonferroni correction on the total effective tests (n=17.7 variables, tested in two – categorical and dimensional – sets of analyses). The resulting multiple-testing adjusted p-value was 0.0014. For VBM analyses, differences were considered significant if they survived cluster-mass thresholding with the FSL easythresh option (www.fmrib.ox.ac.uk/fsl), using an initial cluster forming threshold of z>3.1. We estimated each cluster’s significance level based on Gaussian random-field theory and reported the clusters surviving a family-wise error (FWE)-corrected significance threshold of p<0.05.

RESULTS

Confirmatory analysis: brain measures in all childhood-ADHD probands vs. controls

Preliminary analyses confirmed previous findings (10) of significantly smaller grey matter volume, in individuals with ADHD-C diagnosed at baseline relative to controls (see Table S1 in the online supplement). Relative to controls, childhood-ADHD-C probands further showed smaller grey matter volume in clusters encompassing the precentral gyrus, medial and orbitofrontal cortex, and
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(para)cingulate cortices, similar to (16), as well as smaller volume in one opercular/insular cortex cluster and greater volume in a small brainstem cluster (see Table S1 in the online supplement).

Brain measures and current diagnostic status

Whole-Brain and Subcortical Volumes: No significant group effects emerged for white matter volume. Effects of current diagnostic status on total, grey matter, total accumbens, total amygdala and left putamen volumes did not survive correction for multiple testing (Table 2). A significant overall effect of current diagnostic status emerged for the amygdala volume in the left hemisphere, but not in the remaining subcortical regions (Table 2). Using multiple-testing corrected p-values, post-hoc comparisons indicated significantly greater left amygdala volume than in controls for ADHD full-persisters (β=0.13; CI=0.07-.19; p<0.001), but not partial-persisters (β=0.03; CI=-0.03,-0.10; p=0.35) or remitters (β=0.05; CI=-0.05,-0.15; p=0.32); the remaining comparisons did not survive multiple-testing correction (all β≤0.10, all p≥0.004).

Voxel-Based Morphometry: ADHD full-persisters showed significantly lower grey matter volume relative to controls in seven clusters. These clusters encompassed areas in the frontal pole, temporal and orbitofrontal cortices, paracingulate and posterior cingulate cortices (Table 3). ADHD partial-persisters had significantly smaller grey matter volumes than controls in five clusters (Table 3); two of these were located in the cuneal and lateral occipital cortices, and the remaining encompassed the frontal pole, precentral gyrus and paracingulate cortex. Analyses revealed four clusters in which ADHD-remitters had significantly smaller grey matter volumes than controls; these were located in the left lateral occipital cortex, bilateral lingual gyrus and left precuneus, as well as in the right planum polare/superior temporal gyrus (Table 3). The comparisons between ADHD full-persisters and partial-persisters did not yield significant results. Relative to ADHD-remitters, full-persisters and
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partial-persisters showed, respectively, greater grey matter volume in a cluster located in the left occipital pole and in one cluster located in the left precuneus; smaller grey matter volume emerged in ADHD full-persisters relative to remitters in a cluster including the left precentral gyrus (Table 3). Finally, VBM analysis identified one cluster, located in the brainstem, in which ADHD full-persisters had significantly greater grey matter volume than controls (Table 3). Figure S1 in the online supplement displays the mean voxel values for each group in all identified clusters.

Brain measures and ADHD symptom change

Within individuals with a childhood-ADHD diagnosis, the ADHD symptom change was not significantly associated with whole-brain or subcortical volumes (Table 4). ADHD symptom reduction over time was significantly positively associated with the volume of three clusters, indicating greater that grey matter volume in these areas related to greater ADHD symptom reduction over time; these clusters were located in the left superior frontal gyrus, left precentral gyrus and left cerebellum (Table 4; Figure 1). The volume of one cluster located in the right middle-frontal gyrus was significantly negatively associated with the ADHD symptom reduction; smaller gray matter volume in this area was associated with greater reduction of ADHD symptoms (Table 4; Figure 1).

Potential confounders on brain measures associations

Age did not show a significant association with the volumes of the left amygdala, the clusters identified in VBM categorical analysis or in VBM dimensional analysis (see Table S2 in the online supplement). The lower grey matter volume in the orbitofrontal/temporal cluster in ADHD full-persisters, relative to controls, was sex-dependent (see Table S2 in the online supplement): a significant difference between full-persisters and controls in this cluster was present in males (β=-0.059; CI=-0.081,-0.037; p<0.001), but not in females (β=-0.001; CI=-0.031,0.029; p=0.94). Sex did
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not influence the remaining brain alterations emerging in the main analyses (see Table S2 in the online supplement). When controlling for IQ, results for the volumes of the left amygdala and the clusters identified in VBM analyses remained significant (see Table S3 in the online supplement). Finally, when controlling for lifetime use of ADHD medications, results of the VBM categorical and dimensional analyses remained unchanged (see Table S4 in the online supplement).

DISCUSSION

Exploiting data from the largest sample to date with longitudinal information on ADHD persistence/remission and cross-sectional MRI data at follow-up, this study substantially advances knowledge on reduced grey matter volumes in individuals with ADHD (10, 16) by taking into account the course of ADHD over 6 years and defining persistence/remission both with a categorical and a dimensional approach. The VBM analysis revealed brain structure differences between ADHD full-persisters, partial-persisters, and remitters versus controls, and associations of brain measures with symptom change over time. Specifically, lower volume in clusters located in prefrontal, frontal, precentral and cerebellar regions were associated with the persistence of an ADHD diagnosis and/or with a smaller ADHD symptom decline over time. Conversely, lower volume in visual/auditory cortices emerged in relation to ADHD remission. Analysis of anatomically-defined brain clusters revealed that ADHD full-persisters had significantly larger left amygdala volumes relative to controls. Medication use, age, sex and IQ did not have an influence on the results.

We found that all individuals with childhood ADHD-C, regardless of current diagnostic status, showed smaller grey matter volume in the frontal poles, precentral gyri and paracingulate cortex compared to controls. Reductions in these areas largely overlapped with those reported previously in individuals with a current diagnosis of ADHD in this sample (16), and resemble those commonly
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associated with ADHD in VBM (32) and cortical thickness (33, 34) studies. At follow-up, however, significant volume differences from controls emerged in more clusters in the persistent ADHD subgroups (full-persisters and partial-persisters) than in ADHD-remitters. This pattern of results may be partly due to the smaller sample size in the remittent group. Yet, it suggests that individuals with persistent impairments in one or both ADHD subdomains show the most prominent atypicalities in brain regions that are jointly involved in processes typically impaired in ADHD, such as attention, executive functioning and visual-motor coordination (6).

Conversely, ADHD persistence and remission were distinguished by subtle grey matter characteristics. The largest effects emerged as lower volume in clusters located in the left precentral gyrus, superior-frontal gyrus and left cerebellum in relation to ADHD persistence in categorical and/or dimensional VBM analyses within childhood-ADHD individuals. These findings are in line with evidence from longitudinal studies showing that, during the expected loss of grey matter in frontal cortices from childhood to young adulthood (35), cortical development in ADHD remitters converges toward typical maturation (19, 20). Moreover, lower volume in cerebellar regions was observed in ADHD persisters versus remitters in the only other VBM study on ADHD persistence/remission (26). Therefore, our findings support existing models on normalization of fronto-cerebellar networks, among others, in ADHD remission (8, 21).

Small volumes in visual and auditory brain areas characterized ADHD remission, but not persistence, as indicated by lower grey matter volume in middle/lateral occipital cortices in ADHD-remitters and partial-persisters (those with complete and partial remission, respectively) and in the superior-temporal/Heschl’s gyrus in remitters relative to controls. Structural differences in visual cortices previously emerged in ADHD persisters, but were not significant in remitters (26). Regarding the
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primary auditory cortex, while structural differences were not reported in either persisters or remitters in Proal et al. (26), smaller Heschl’s gyrus volume was observed in children with ADHD relative to controls in another study (36). The relevance of structural and functional abnormalities in sensory systems in ADHD has been increasingly discussed (37). A recent meta-analysis of functional MRI studies indicated that visual and auditory networks are over-activated in individuals with ADHD compared to controls during cognitive performance (38), suggesting that individuals with ADHD might rely on lower-level sensory systems to compensate for impaired function of frontal-control networks (8). The association of lower grey matter volume in the right middle-frontal gyrus with greater ADHD symptom decline found here may further be in line with this compensatory hypothesis. Indeed, the middle-frontal gyrus has been thought to be a key node in the ventral attention network, which was found to be activated during reorienting of attention following presentation of unexpected visual stimuli that might be relevant to the specific task (39).

The main finding of the subcortical volumes analysis is the larger left amygdala volume in ADHD full-persisters, but not in partial-persisters and remitters, relative to controls. While previous evidence mostly suggests reduced amygdala volumes in ADHD (7, 40), prior studies included individuals with ADHD diagnosed at one point in time only. In contrast, the ADHD persisters in the current study were more stringently defined by meeting full criteria for combined-type ADHD at two time points. Smaller amygdala volumes have not consistently been reported in youths and adults with ADHD and were predominantly identified in samples of untreated individuals (15). Structural alterations in the amygdala are of interest given the acknowledged association of ADHD with emotional dysregulation (17). Future studies are warranted to examine the relationship of amygdala with emotional regulation deficits such as those seen in anxiety, depression and autism in the context of ADHD persistence and remission.
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The comprehensive investigation of brain volumes, with both a priori and voxel-based measures, and the use of two assessments over a 6-year clinical follow-up to examine the course of ADHD are strengths of the current study. However, some study limitations should be acknowledged. First, we investigated the structural brain correlates of ADHD persistence/remission using a cross-sectional MRI study design, limiting our ability to draw conclusions on whether within-individual structural brain changes over time link to symptom decline over time. The hypothesized relationships between brain volumetric normalizations and decline of ADHD symptoms or diagnosis need to be tested in future longitudinal MRI studies. Second, the number of individuals with childhood DSM-IV Combined ADHD who were in remission 6 years later was small, as was the average ADHD symptom decline over time. This may partly explain the finding of only subtle associations of brain measures with ADHD persistence/remission and symptom decline.

In sum, reduced grey matter volumes in regions involved in top-down behavior control (frontal-cerebellar cortices) appear to be key in the persistence of ADHD diagnosis or symptoms, while the association of ADHD remission with reduced volumes in visual/auditory cortices suggests possible earlier maturation in these areas in ADHD remitters. While awaiting confirmation from longitudinal MRI studies, this observation supports previous theories that over-reliance on sensory processing might compensate for frontal-control deficits in ADHD (38).
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REFERENCES


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### TABLES

**Table 1. Participant characteristics**

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<td>53 (36%)</td>
<td>&lt;0.001</td>
<td>ADHD-FP = ADHD-PP = ADHD-R &lt; C</td>
</tr>
<tr>
<td>Age in years, mean (SD)</td>
<td>17.8 (2.8)</td>
<td>17.9 (2.7)</td>
<td>17.5 (2.4)</td>
<td>16.9 (3.1)</td>
<td>0.07</td>
<td>-</td>
</tr>
<tr>
<td>IQ, mean (SD)</td>
<td>94.6 (15.1)</td>
<td>96.8 (15.6)</td>
<td>96.6 (12.0)</td>
<td>109.1 (13.7)</td>
<td>&lt;0.001</td>
<td>ADHD-FP = ADHD-PP = ADHD-R &lt; C</td>
</tr>
<tr>
<td>Clinical Interview at follow-up(^a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADHD symptoms</td>
<td>15.5 (1.9)</td>
<td>11.6 (2.5)</td>
<td>5.9 (3.2)</td>
<td>0.7 (2.0)</td>
<td>&lt;0.001</td>
<td>ADHD-FP &gt; ADHD-PP &gt; ADHD-R &gt; C</td>
</tr>
<tr>
<td>Clinical Interview at baseline(^a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADHD symptoms</td>
<td>16.5 (2.0)</td>
<td>16.1 (2.1)</td>
<td>16.0 (2.0)</td>
<td>16.0 (2.0)</td>
<td>-</td>
<td>0.78</td>
</tr>
<tr>
<td>Medication use (ever)(^b), n (%)</td>
<td>96 (96%)</td>
<td>95 (90%)</td>
<td>28 (88%)</td>
<td>-</td>
<td>0.14</td>
<td>-</td>
</tr>
</tbody>
</table>

ADHD, attention-deficit/hyperactivity disorder; ADHD-FP, attention-deficit/hyperactivity disorder full-persisters; ADHD-PP, attention-deficit/hyperactivity disorder partial-persisters; ADHD-R, attention-deficit/hyperactivity disorder remitters; C, Control individuals; IQ, intelligence quotient (IQ was not available for one full-persister, one partial-persister and two controls at follow-up); SD, standard deviation of the group mean. \(^a\)Total ADHD symptom count; maximal eighteen symptoms. \(^b\)Information on lifetime use of psychoactive medications for ADHD was gathered from pharmacy transcripts and questionnaire reports. Group differences on sex and lifetime use of ADHD medications were tested via chi-square test; group differences on age, IQ and ADHD symptom scores were tested with regression models.
### Grey matter volumes in ADHD persistence/remission

**Table 2.** Brain volumetric measures in ADHD full-persisters, ADHD partial-persisters, ADHD remitters and control individuals

<table>
<thead>
<tr>
<th>Brain region</th>
<th>ADHD-FP n=100</th>
<th>ADHD-PP n=106</th>
<th>ADHD-R n=32</th>
<th>Controls n=147</th>
<th>Group comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
<td>F p</td>
</tr>
<tr>
<td><strong>Total Brain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grey Matter</td>
<td>1262.71 (11.26)</td>
<td>1250.81 (9.8)</td>
<td>1251.75 (21.4)</td>
<td>1294.29 (9.76)</td>
<td>3.20 0.02</td>
</tr>
<tr>
<td></td>
<td>736.55 (6.52)</td>
<td>734.95 (5.76)</td>
<td>730.11 (13.4)</td>
<td>764.54 (5.95)</td>
<td>4.66 0.003</td>
</tr>
<tr>
<td>White Matter</td>
<td>526.15 (5.95)</td>
<td>515.86 (5.3)</td>
<td>521.64 (9.94)</td>
<td>529.75 (5.11)</td>
<td>1.20 0.31</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Accumbens, total</strong></td>
<td>1.17 (0.02)</td>
<td>1.16 (0.02)</td>
<td>1.14 (0.02)</td>
<td>1.13 (0.02)</td>
<td>2.87 0.04</td>
</tr>
<tr>
<td>left hemisphere</td>
<td>0.62 (0.01)</td>
<td>0.62 (0.01)</td>
<td>0.61 (0.01)</td>
<td>0.61 (0.01)</td>
<td>1.55 0.20</td>
</tr>
<tr>
<td>right hemisphere</td>
<td>0.55 (0.01)</td>
<td>0.54 (0.01)</td>
<td>0.53 (0.01)</td>
<td>0.53 (0.01)</td>
<td>3.14 0.03</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Amygdala, total</strong></td>
<td>2.74 (0.05)</td>
<td>2.63 (0.04)</td>
<td>2.66 (0.08)</td>
<td>2.63 (0.03)</td>
<td>2.65 0.05</td>
</tr>
<tr>
<td>left hemisphere</td>
<td>1.36 (0.02)</td>
<td>1.25 (0.03)</td>
<td>1.27 (0.05)</td>
<td>1.25 (0.02)</td>
<td>6.34 &lt;0.001</td>
</tr>
<tr>
<td>right hemisphere</td>
<td>1.39 (0.03)</td>
<td>1.38 (0.03)</td>
<td>1.38 (0.05)</td>
<td>1.38 (0.02)</td>
<td>0.32 0.81</td>
</tr>
<tr>
<td></td>
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<td></td>
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</tr>
<tr>
<td><strong>Caudate Nucleus, total</strong></td>
<td>8.01 (0.1)</td>
<td>7.97 (0.09)</td>
<td>8.04 (0.17)</td>
<td>8.28 (0.08)</td>
<td>0.43 0.73</td>
</tr>
<tr>
<td>left hemisphere</td>
<td>3.93 (0.05)</td>
<td>3.91 (0.05)</td>
<td>3.95 (0.09)</td>
<td>4.06 (0.04)</td>
<td>0.34 0.80</td>
</tr>
<tr>
<td>right hemisphere</td>
<td>4.09 (0.05)</td>
<td>4.06 (0.05)</td>
<td>4.09 (0.09)</td>
<td>4.22 (0.04)</td>
<td>0.37 0.77</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Globus pallidus, total</strong></td>
<td>3.81 (0.04)</td>
<td>3.78 (0.03)</td>
<td>3.76 (0.06)</td>
<td>3.78 (0.03)</td>
<td>2.28 0.08</td>
</tr>
<tr>
<td>left hemisphere</td>
<td>1.88 (0.02)</td>
<td>1.86 (0.02)</td>
<td>1.85 (0.03)</td>
<td>1.87 (0.02)</td>
<td>1.85 0.14</td>
</tr>
<tr>
<td>right hemisphere</td>
<td>1.93 (0.02)</td>
<td>1.91 (0.02)</td>
<td>1.9 (0.03)</td>
<td>1.91 (0.02)</td>
<td>2.20 0.09</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hippocampus, total</strong></td>
<td>7.9 (0.08)</td>
<td>7.77 (0.07)</td>
<td>7.74 (0.13)</td>
<td>7.92 (0.08)</td>
<td>0.47 0.70</td>
</tr>
<tr>
<td>left hemisphere</td>
<td>3.88 (0.04)</td>
<td>3.81 (0.04)</td>
<td>3.8 (0.07)</td>
<td>3.89 (0.04)</td>
<td>0.46 0.71</td>
</tr>
<tr>
<td>right hemisphere</td>
<td>4.02 (0.05)</td>
<td>3.96 (0.04)</td>
<td>3.94 (0.07)</td>
<td>4.03 (0.04)</td>
<td>0.32 0.81</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Putamen, total</strong></td>
<td>10.99 (0.11)</td>
<td>10.91 (0.11)</td>
<td>10.98 (0.16)</td>
<td>10.91 (0.09)</td>
<td>2.15 0.09</td>
</tr>
<tr>
<td>left hemisphere</td>
<td>5.5 (0.06)</td>
<td>5.46 (0.06)</td>
<td>5.56 (0.08)</td>
<td>5.46 (0.05)</td>
<td>2.63 0.05</td>
</tr>
<tr>
<td>right hemisphere</td>
<td>5.48 (0.06)</td>
<td>5.45 (0.05)</td>
<td>5.42 (0.08)</td>
<td>5.44 (0.05)</td>
<td>1.78 0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Thalamus, total</strong></td>
<td>17.08 (0.16)</td>
<td>16.81 (0.15)</td>
<td>16.87 (0.27)</td>
<td>17.12 (0.14)</td>
<td>1.07 0.36</td>
</tr>
<tr>
<td>left hemisphere</td>
<td>8.62 (0.08)</td>
<td>8.5 (0.08)</td>
<td>8.56 (0.14)</td>
<td>8.65 (0.07)</td>
<td>0.96 0.41</td>
</tr>
<tr>
<td>right hemisphere</td>
<td>8.46 (0.08)</td>
<td>8.31 (0.07)</td>
<td>8.31 (0.13)</td>
<td>8.47 (0.07)</td>
<td>1.23 0.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Brainstem, total</strong></td>
<td>22.35 (0.24)</td>
<td>22.25 (0.24)</td>
<td>22.23 (0.47)</td>
<td>22.4 (0.19)</td>
<td>1.47 0.22</td>
</tr>
</tbody>
</table>

Means are based on estimated marginal means corrected for age, age$^2$, sex, and scanner location; for subcortical volumes, correction for total brain volume is also included. ADHD-FP, attention-deficit/hyperactivity disorder full-persisters; ADHD-PP, attention-deficit/hyperactivity disorder partial-persisters; ADHD-R, attention-deficit/hyperactivity disorder remitters. $^a$Sum of total grey and white matter volumes. Degrees of freedom ranged between df=3,288 and df=3,291. Boldface indicates that the P value remains significant following multiple testing (effective number-adjusted P-value threshold of 0.0014).
Table 3. Clusters of grey matter showing voxel-based morphometry differences between groups defined by diagnostic status at follow-up

<table>
<thead>
<tr>
<th>Cluster area (hemisphere)</th>
<th>Cluster size (No. voxels)</th>
<th>MNI coordinates (x, y, z)</th>
<th>Best z value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ADHD-FP &lt; Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal Pole (R)</td>
<td>806</td>
<td>39, 51, -9</td>
<td>4.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Frontal Pole (L)</td>
<td>1259</td>
<td>-46, 41, 33</td>
<td>4.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Frontal Pole (L)</td>
<td>303</td>
<td>-30, 53, -4</td>
<td>4.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Paracingulate gyrus, superior frontal gyrus (L)</td>
<td>1706</td>
<td>-15, 53, 11</td>
<td>4.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Posterior cingulate cortex (L)</td>
<td>236</td>
<td>-13, -37, 30</td>
<td>3.92</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Superior frontal gyrus, precentral gyrus (R)</td>
<td>250</td>
<td>21, 3, 51</td>
<td>3.75</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Temporal pole, orbitofrontal cortex (L)</td>
<td>554</td>
<td>-25, 13, -28</td>
<td>4.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>ADHD-PP &lt; Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuneous (L)</td>
<td>200</td>
<td>-12, -75, 23</td>
<td>3.65</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Frontal Pole (R)</td>
<td>528</td>
<td>39, 45, -6</td>
<td>3.75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lateral occipital cortex (R)</td>
<td>328</td>
<td>57, -72, 30</td>
<td>4.16</td>
<td>0.001</td>
</tr>
<tr>
<td>Paracingulate gyrus, superior frontal gyrus (L)</td>
<td>1741</td>
<td>-11, 53, 18</td>
<td>4.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Precentral gyrus (L)</td>
<td>577</td>
<td>-41, -11, 53</td>
<td>4.21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>ADHD-R &lt; Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral occipital cortex (L)</td>
<td>110</td>
<td>-54, -73, 9</td>
<td>4.11</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Lingual gyrus (R)</td>
<td>93</td>
<td>10, -72, 2</td>
<td>4.00</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Lingual gyrus, precuneus cortex (L)</td>
<td>90</td>
<td>-5, -60, 9</td>
<td>3.69</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Planum polare, Heschl's gyrus, superior temporal gyrus (R)</td>
<td>164</td>
<td>51, -4, -3</td>
<td>3.45</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>ADHD-FP &lt; ADHD-R</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precentral gyrus (L)</td>
<td>47</td>
<td>-9, -21, 81</td>
<td>3.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>ADHD-FP &gt; Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brainstem</td>
<td>60</td>
<td>-5, -21, -21</td>
<td>3.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>ADHD-PP &gt; ADHD-R</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precuneus cortex (L)</td>
<td>27</td>
<td>-15, -57, 37</td>
<td>3.77</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>ADHD-FP &gt; ADHD-R</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occipital pole (L)</td>
<td>143</td>
<td>-12, -99, -16</td>
<td>3.49</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Clusters represent areas with significant group differences emerging from the voxel-based cluster robust regression, controlling for sex, age at follow-up, follow-up duration and scanner location. For each cluster, the table lists the cluster’s anatomical area label, cluster size as the number of voxels and the stereotaxic coordinates for the peak voxel in Montreal Neurological Institute (MNI) coordinates. L: left hemisphere; R: right hemisphere. ADHD-FP, attention-deficit/hyperactivity disorder full-persisters; ADHD-PP, attention-deficit/hyperactivity disorder partial-persisters; ADHD-R, attention-deficit/hyperactivity disorder remitters.
Whole-brain alterations in life-time ADHD.

Table 4. Association of brain volumetric measures with the change in total ADHD symptoms over time

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Change in ADHD symptoms</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β [95% CI]</td>
<td></td>
</tr>
<tr>
<td>Total Brain</td>
<td>0.38 [-3.59, 4.34]</td>
<td>0.85</td>
</tr>
<tr>
<td>Grey Matter</td>
<td>0.21 [-2.16, 2.58]</td>
<td>0.86</td>
</tr>
<tr>
<td>White Matter</td>
<td>0.17 [-1.75, 2.09]</td>
<td>0.86</td>
</tr>
<tr>
<td>Accumbens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>left hemisphere</td>
<td>0.00 [-0.01, 0.01]</td>
<td>0.72</td>
</tr>
<tr>
<td>right hemisphere</td>
<td>0.00 [-0.01, 0.00]</td>
<td>0.61</td>
</tr>
<tr>
<td>Amygdala</td>
<td></td>
<td></td>
</tr>
<tr>
<td>left hemisphere</td>
<td>-0.01 [-0.04, 0.01]</td>
<td>0.33</td>
</tr>
<tr>
<td>right hemisphere</td>
<td>0.00 [-0.02, 0.02]</td>
<td>0.97</td>
</tr>
<tr>
<td>Caudate Nucleus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>left hemisphere</td>
<td>-0.01 [-0.05, 0.03]</td>
<td>0.53</td>
</tr>
<tr>
<td>right hemisphere</td>
<td>0.00 [-0.02, 0.01]</td>
<td>0.70</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>left hemisphere</td>
<td>-0.01 [-0.02, 0.01]</td>
<td>0.22</td>
</tr>
<tr>
<td>right hemisphere</td>
<td>0.00 [-0.01, 0.00]</td>
<td>0.32</td>
</tr>
<tr>
<td>Hippocampus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>left hemisphere</td>
<td>-0.01 [-0.05, 0.01]</td>
<td>0.12</td>
</tr>
<tr>
<td>right hemisphere</td>
<td>-0.01 [-0.03, 0.01]</td>
<td>0.27</td>
</tr>
<tr>
<td>Putamen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>left hemisphere</td>
<td>0.00 [-0.05, 0.04]</td>
<td>0.86</td>
</tr>
<tr>
<td>right hemisphere</td>
<td>0.00 [-0.02, 0.03]</td>
<td>0.85</td>
</tr>
<tr>
<td>Thalamus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>left hemisphere</td>
<td>-0.02 [-0.07, 0.04]</td>
<td>0.58</td>
</tr>
<tr>
<td>right hemisphere</td>
<td>0.00 [-0.03, 0.03]</td>
<td>0.91</td>
</tr>
<tr>
<td>Brainstem</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.03 [-0.08, 0.15]</td>
<td>0.56</td>
</tr>
</tbody>
</table>

For each cluster, the table lists the cluster's anatomical area label, cluster size as the number of voxels and the stereotaxic coordinates for the peak voxel in Montreal Neurological Institute (MNI) coordinates. L: left hemisphere; R: right hemisphere.

*a Results from the regression models predicting volumetric brain measures as a function of the difference in total ADHD symptoms scores between baseline to follow-up. A negative regression coefficient reflects a decrease in mean brain volumes (in milliliters) for each unit of reduction in symptoms. b Clusters represent areas with significant association with ADHD symptom change emerging within the individuals with ADHD-C in childhood. For each cluster, the table lists the cluster's anatomical area label, cluster size as the number of voxels and the stereotaxic coordinates for the peak voxel in Montreal Neurological Institute (MNI) coordinates. L: left hemisphere; R: right hemisphere.
Figure 2. Associations of change in total ADHD symptoms over time and mean voxel value for the identified clusters within the individuals with ADHD established in childhood (n = 238).
Adamo et al., Grey matter volume relates to persistence and remission of ADHD symptoms and diagnosis

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- **Supplement 1.** Diagnostic Assessments of ADHD at baseline and follow-up assessments
- **Table S1.** Brain volumetric measures and voxel-based morphometry in all participants with a childhood ADHD diagnosis and control individuals
- **Table S2.** Effects of age and sex on associations of brain volumes with diagnostic status at follow-up or ADHD symptoms change over time
- **Table S3.** Associations of grey matter volumes with diagnostic status at follow up or ADHD symptom change controlling for IQ in the individuals with childhood ADHD
- **Table S4.** Associations of grey matter volumes with diagnostic status at follow up or ADHD symptom change controlling for lifetime use of medications in the individuals with childhood ADHD
- **Figure S1.** Mean voxel values for the clusters showing significant voxel-based morphometry differences between groups defined by diagnostic status at follow-up
- **References**
Supplement 1. Diagnostic Assessments of ADHD at baseline and follow-up assessments

During IMAGE, the Conners’ parent- and teacher-rating scales (CPRS-R:L and CTRS-R:L, respectively; 1, 2) and the Strengths and Difficulties Questionnaire (SDQ) (3) were used for initial screening; childhood ADHD diagnosis was established using the Parental Account of Childhood symptoms (PACS) (4, 5).

To determine ADHD status during NeuroIMAGE, participants were assessed using ADHD questionnaires and a semi-structured diagnostic interview. For participants using medication, ratings were done regarding functioning off medication. Each participant was assessed with the CPRS-R:L (1) combined with either a teacher rating (CTRS-R:L) (2), for participants <18 years, or a self-report (CAARS-S:L) (6), for participants ≥18 years. Parents of all participants and participants who were ≥12 years old were administered the Dutch translation of the Schedule for Affective Disorders and Schizophrenia for School-Age Children – Present and Lifetime Version (KSADS-PL) (7) to assess presence/absence of ADHD symptoms and impairments.

A diagnostic algorithm was applied to combine ADHD symptom counts on the interviews and questionnaires, both providing operational definitions of the 18 DSM-IV-defined symptoms (8). Participants were classified as ADHD full-persisters if they presented with ≥6 symptoms of inattention and ≥6 symptoms of hyperactivity/impulsivity, provided they (a) met the DSM-IV impact and duration criteria, (b) had an onset before age 7 and (c) received a standardized T-score ≥63 on at least one DSM-ADHD subscale of the Conners questionnaires. Participants were classified as ADHD partial-persisters when presenting with ≥6 symptoms in one of the ADHD symptom domains (inattentive or hyperactive/impulsive) and met DSM-IV criteria for onset, impact and pervasiveness as described above. For young adults (≥18 years), the adult adapted DSM-5 (9) criteria were used, such that a combined count of 5 symptoms was sufficient for a diagnosis. Participants with ADHD-C at baseline who did not fulfill the above criteria for number of symptoms and impairment at follow-up were classified as ADHD-remitters.
To avoid inclusion of individuals with subclinical ADHD in childhood/adolescence and potential late-onset ADHD (10), we excluded controls with missing ADHD ratings at baseline. At follow-up, controls were required to have T-scores <63 on both ADHD subscales of the Conners questionnaires and ≤3 symptoms derived from the combined symptom counts of the K-SADS and CTRS-R:L/CAARS-S:L.
Table S1. Brain volumetric measures and voxel-based morphometry in all participants with a childhood ADHD diagnosis and control individuals

<table>
<thead>
<tr>
<th>A priori defined brain region</th>
<th>Childhood ADHD (n=238)</th>
<th>Controls (n=147)</th>
<th>Group comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
<td>F</td>
</tr>
<tr>
<td><strong>Total Brain</strong></td>
<td>1255.92 (7.41)</td>
<td>1294.31 (9.73)</td>
<td>8.79</td>
</tr>
<tr>
<td>Grey Matter</td>
<td>734.92 (4.44)</td>
<td>764.61 (5.92)</td>
<td>13.94</td>
</tr>
<tr>
<td>White Matter</td>
<td>521.00 (3.86)</td>
<td>529.69 (5.09)</td>
<td>1.69</td>
</tr>
<tr>
<td><strong>Accumbens, total</strong></td>
<td>1.17 (0.01)</td>
<td>1.11 (0.01)</td>
<td>8.63</td>
</tr>
<tr>
<td>left hemisphere</td>
<td>0.62 (0.01)</td>
<td>0.60 (0.01)</td>
<td>4.45</td>
</tr>
<tr>
<td>right hemisphere</td>
<td>0.52 (0.01)</td>
<td>0.55 (0.01)</td>
<td>9.27</td>
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<tr>
<td><strong>Amygdala, total</strong></td>
<td>2.70 (0.03)</td>
<td>2.60 (0.03)</td>
<td>5.21</td>
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<tr>
<td>left hemisphere</td>
<td>1.31 (0.02)</td>
<td>1.23 (0.02)</td>
<td>8.32</td>
</tr>
<tr>
<td>right hemisphere</td>
<td>1.39 (0.02)</td>
<td>1.36 (0.02)</td>
<td>0.95</td>
</tr>
<tr>
<td><strong>Caudate Nucleus, total</strong></td>
<td>8.07 (0.05)</td>
<td>8.15 (0.07)</td>
<td>0.94</td>
</tr>
<tr>
<td>left hemisphere</td>
<td>3.96 (0.03)</td>
<td>3.99 (0.03)</td>
<td>0.62</td>
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<tr>
<td>right hemisphere</td>
<td>4.11 (0.03)</td>
<td>4.16 (0.04)</td>
<td>0.98</td>
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<tr>
<td><strong>Globus pallidus, total</strong></td>
<td>3.81 (0.02)</td>
<td>3.72 (0.03)</td>
<td>6.72</td>
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<tr>
<td>left hemisphere</td>
<td>1.88 (0.01)</td>
<td>1.84 (0.01)</td>
<td>5.47</td>
</tr>
<tr>
<td>right hemisphere</td>
<td>1.93 (0.01)</td>
<td>1.88 (0.01)</td>
<td>6.53</td>
</tr>
<tr>
<td><strong>Hippocampus, total</strong></td>
<td>7.87 (0.04)</td>
<td>7.84 (0.07)</td>
<td>0.12</td>
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<td>left hemisphere</td>
<td>3.86 (0.03)</td>
<td>3.84 (0.04)</td>
<td>0.02</td>
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<tr>
<td>right hemisphere</td>
<td>4.01 (0.03)</td>
<td>3.99 (0.04)</td>
<td>0.16</td>
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<tr>
<td><strong>Putamen, total</strong></td>
<td>11.03 (0.06)</td>
<td>10.77 (0.08)</td>
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<tr>
<td>left hemisphere</td>
<td>5.53 (0.03)</td>
<td>5.40 (0.04)</td>
<td>6.05</td>
</tr>
<tr>
<td>right hemisphere</td>
<td>5.50 (0.03)</td>
<td>5.37 (0.04)</td>
<td>5.22</td>
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<td><strong>Thalamus, total</strong></td>
<td>17.07 (0.08)</td>
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</tr>
<tr>
<td>left hemisphere</td>
<td>8.63 (0.06)</td>
<td>8.63 (0.04)</td>
<td>1.97</td>
</tr>
<tr>
<td>right hemisphere</td>
<td>8.36 (0.05)</td>
<td>8.44 (0.04)</td>
<td>1.37</td>
</tr>
<tr>
<td><strong>Brainstem, total</strong></td>
<td>22.49 (0.13)</td>
<td>22.06 (0.14)</td>
<td>4.38</td>
</tr>
</tbody>
</table>

Cluster area (hemisphere) showing VBM case-control differences

<table>
<thead>
<tr>
<th>Clusters</th>
<th>Cluster size (No. voxels)</th>
<th>MNI coordinates (x, y, z)</th>
<th>Best z value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>childhood ADHD &lt; Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal Pole (R)</td>
<td>1412</td>
<td>41, 50, -9</td>
<td>4.48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Frontal Pole (L)</td>
<td>1095</td>
<td>-46, 41, 33</td>
<td>4.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Frontal Pole (L)</td>
<td>504</td>
<td>-31, 53, -3</td>
<td>4.24</td>
<td>0.001</td>
</tr>
<tr>
<td>Paracingulate gyrus, anterior cingulate cortex (L)</td>
<td>1709</td>
<td>-5, 39, -5</td>
<td>3.86</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anterior cingulate cortex, extending to the posterior cingulate (L)</td>
<td>333</td>
<td>-8, -11, 40</td>
<td>3.89</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Posterior cingulate cortex (R)</td>
<td>398</td>
<td>9, -30, 35</td>
<td>4.09</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Orbitofrontal cortex, temporal pole (L)</td>
<td>531</td>
<td>-25, 15, -27</td>
<td>3.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cluster area (hemisphere) showing VBM case-control differences</td>
<td>Cluster size (No. voxels)</td>
<td>MNI coordinates (x, y, z)</td>
<td>Best z value</td>
<td>p</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
<td>--------------------------</td>
<td>---------------------------</td>
<td>-------------</td>
<td>-----</td>
</tr>
<tr>
<td>Paracingulate gyrus, superior frontal gyrus (L)</td>
<td>2876</td>
<td>-11, 53, 17</td>
<td>4.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Precentral gyrus (L)</td>
<td>526</td>
<td>-36, -11, 54</td>
<td>3.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Central Opercular Cortex, extending to Insula (R)</td>
<td>324</td>
<td>38, 0, 18</td>
<td>3.60</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Brainstem</td>
<td>35</td>
<td>-5, -21, -22</td>
<td>3.74</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

In the top part of the table, means are based on estimated marginal means corrected for age, age², sex, and scanner location; for subcortical volumes, correction for total brain volume is also included. ADHD: attention-deficit/hyperactivity disorder. *Sum of total grey and white matter volumes. As outlier total brain and subcortical volumes exceeding 3 SDs from the sample mean were removed, degrees of freedom ranged between df=3,288 and df=3,291. Boldface indicates that the P value remains significant following multiple testing (effective number-adjusted P-value threshold of 0.0014). In the bottom part of the table, clusters represent areas with significant group differences emerging from the voxel-based cluster robust regression, controlling for sex, age at follow-up, follow-up duration and scanner location. For each cluster, the table lists the cluster’s anatomical area label, cluster size as the number of voxels and the stereotaxic coordinates for the peak voxel in Montreal Neurological Institute (MNI) coordinates. L: left hemisphere; R: right hemisphere; VBM: voxel-based morphometry.
Table S2. Effects of age and sex on associations of brain volumes with diagnostic status at follow-up or ADHD symptoms change over time

<table>
<thead>
<tr>
<th>A priori defined brain region (hemisphere)</th>
<th>age x diagnosis/</th>
<th>age(^2) x diagnosis/</th>
<th>sex x diagnosis/</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>age change p</td>
<td>age change p</td>
<td>symptom change p</td>
</tr>
<tr>
<td>Amygdala volume (L)</td>
<td>0.73</td>
<td>0.77</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Cluster area (hemisphere)

**ADHD-FP < Controls**
- Frontal Pole (R): 0.50, 0.56, 0.56
- Frontal Pole (L): 0.52, 0.62, 0.76
- Frontal Pole (L): 0.63, 0.65, 0.69
- Paracingulate gyrus, superior frontal gyrus (L): 0.90, 0.95, 0.82
- Posterior cingulate cortex (L): 0.89, 0.97, 0.92
- Superior frontal gyrus (R): 0.25, 0.26, 0.82
- Temporal pole, orbitofrontal cortex (L): 0.40, 0.46, **0.002**

**ADHD-PP < Controls**
- Cuneous (L): 0.65, 0.64, 0.77
- Frontal Pole (R): 0.82, 0.73, 0.99
- Lateral occipital cortex (R): 0.11, 0.10, 0.12
- Paracingulate gyrus, superior frontal gyrus (L): 0.44, 0.36, 0.20
- Precentral gyrus (L): 0.08, 0.06, 0.88

**ADHD-R < Controls**
- Lateral occipital cortex (L): 0.41, 0.51, 0.15
- Lingual gyrus (R): 0.79, 0.72, 0.13
- Lingual gyrus, precuneous cortex (L): 0.48, 0.45, 0.80
- Planum polare, Helschl’s gyrus, superior temporal gyrus (R): 0.21, 0.18, 0.52

**ADHD-FP < ADHD-R**
- Precentral gyrus (L): 0.59, 0.61, 0.41

**ADHD-PP > ADHD-R**
- Brainstem: 0.01, 0.01, 0.77

**ADHD-FP > ADHD-R**
- Precuneous cortex (L): 0.60, 0.54, 0.89
- Occipital pole (L): 0.84, 0.88, 0.97

Low volume in relation to greater ADHD symptoms change
- Middle Frontal Gyrus (R): 0.21, 0.21, 0.50

High volume associated with greater ADHD symptoms change
- Superior Frontal Gyrus (L): 0.93, 0.92, 0.06
- Precentral Gyrus (L): 0.20, 0.19, 0.76
- Cerebellum, Posterior lobe (L): 0.74, 0.70, 0.58

Note: uncorrected p-values for the interaction effects of age and age\(^2\), as well as sex with current diagnostic status for the left amygdala volume and the voxel-based morphometry clusters showing a significant association with current diagnostic status and ADHD symptom change, respectively, in the main analysis. L: left hemisphere; R: right hemisphere. Significant interaction effects are indicated in boldface.
Table S3. Associations of grey matter volumes with diagnostic status at follow up or ADHD symptom change controlling for IQ in the individuals with childhood ADHD controlling for IQ

<table>
<thead>
<tr>
<th><strong>A priori defined brain region</strong></th>
<th><strong>p</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdala volume (L)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Cluster area (hemisphere)</strong></th>
<th><strong>p</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ADHD-FP &lt; Controls</strong></td>
<td></td>
</tr>
<tr>
<td>Frontal Pole (R)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Frontal Pole (L)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Frontal Pole (L)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Paracingulate gyrus, superior frontal gyrus (L)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Posterior cingulate cortex (L)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Superior frontal gyrus (R)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temporal pole, orbitofrontal cortex (L)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>ADHD-PP &lt; Controls</strong></td>
<td></td>
</tr>
<tr>
<td>Cuneous (L)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Frontal Pole (R)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lateral occipital cortex (R)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Paracingulate gyrus, superior frontal gyrus (L)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Precentral gyrus (L)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>ADHD-R &lt; Controls</strong></td>
<td></td>
</tr>
<tr>
<td>Lateral occipital cortex (L)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lingual gyrus (R)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lingual gyrus, precuneous cortex (L)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Planum polare, Helschl's gyrus, superior temporal gyrus (R)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>ADHD-FP &lt; ADHD-R</strong></td>
<td></td>
</tr>
<tr>
<td>Precentral gyrus (L)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>ADHD-FP &gt; Controls</strong></td>
<td></td>
</tr>
<tr>
<td>Brainstem</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>ADHD-PP &gt; ADHD-R</strong></td>
<td></td>
</tr>
<tr>
<td>Precuneous cortex (L)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>ADHD-FP &gt; ADHD-R</strong></td>
<td></td>
</tr>
<tr>
<td>Occipital pole (L)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Low volume in relation to greater ADHD symptoms change**
| Middle Frontal Gyrus (R)      | <0.001|

**High volume associated with greater ADHD symptoms change**
| Superior Frontal Gyrus (L)    | <0.001|
| Precentral Gyrus (L)          | <0.001|
| Cerebellum, Posterior lobe (L)| <0.001|

Note: uncorrected p-values for the associations of left amygdala volume and the voxel-based morphometry clusters with current diagnostic status and ADHD symptom change over time, respectively, controlling for IQ. L: left hemisphere; R: right hemisphere. In (a), significant interaction effects are indicated in boldface.
### Table S4. Associations of grey matter volumes with diagnostic status at follow up or ADHD symptom change controlling for lifetime use of medications in the individuals with childhood ADHD

<table>
<thead>
<tr>
<th>Cluster area (hemisphere)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ADHD-FP &lt; ADHD-R</strong></td>
<td></td>
</tr>
<tr>
<td>Precentral gyrus (L)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>ADHD-PP &gt; ADHD-R</strong></td>
<td></td>
</tr>
<tr>
<td>Precuneous cortex (L)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>ADHD-FP &gt; ADHD-R</strong></td>
<td></td>
</tr>
<tr>
<td>Occipital pole (L)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Low volume in relation to greater ADHD symptoms change</strong></td>
<td></td>
</tr>
<tr>
<td>Middle Frontal Gyrus (R)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>High volume associated with greater ADHD symptoms change</strong></td>
<td></td>
</tr>
<tr>
<td>Superior Frontal Gyrus (L)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Precentral Gyrus (L)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cerebellum, Posterior lobe (L)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Note: uncorrected p-values for the associations of the clusters identified in the main analysis with current diagnostic status or ADHD symptoms change, controlling for lifetime use of ADHD medication. L: left hemisphere; R: right hemisphere.
Figure S1.

<table>
<thead>
<tr>
<th></th>
<th>ADHD-FP &lt; Controls</th>
<th>ADHD-PP &lt; Controls</th>
<th>ADHD-R &lt; Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal pole (R)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal pole (L)</td>
<td></td>
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<td></td>
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<tr>
<td>Cuneous (L)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Lateral occipital cortex (L)</td>
<td></td>
<td></td>
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<tr>
<td>Frontal pole (L)</td>
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</tr>
<tr>
<td>Lateral occipital cortex (R)</td>
<td></td>
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</tr>
<tr>
<td>Lyngual gyrus (R)</td>
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<tr>
<td>Paracingulate gyrus, superior frontal gyrus (L)</td>
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<tr>
<td>Posterior cingulate cortex (L)</td>
<td></td>
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<tr>
<td>Precentral gyrus (L)</td>
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<tr>
<td>Superior frontal gyrus (R)</td>
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<td></td>
</tr>
<tr>
<td>Temporal pole, orbitofrontal cortex (L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
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<td></td>
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<tr>
<td>ADHD-R</td>
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<td>ADHD-PP</td>
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Figure S1. Cont’d

<table>
<thead>
<tr>
<th></th>
<th>ADHD-FP &gt; ADHD-R</th>
<th>ADHD-PP &gt; ADHD-R</th>
<th>ADHD-FP &lt; ADHD-R</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ocipital pole (L)</strong></td>
<td>0.35 ± 0.05</td>
<td>0.30 ± 0.04</td>
<td>0.25 ± 0.03</td>
</tr>
<tr>
<td><strong>Precuneous (L)</strong></td>
<td>0.28 ± 0.03</td>
<td>0.23 ± 0.02</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td><strong>Precentral gyrus (L)</strong></td>
<td>0.21 ± 0.01</td>
<td>0.16 ± 0.00</td>
<td>0.11 ± 0.00</td>
</tr>
</tbody>
</table>

ADHD-FP > Controls

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>ADHD-R</th>
<th>ADHD-PP</th>
<th>ADHD-FP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brainstem</strong></td>
<td>0.15 ± 0.04</td>
<td>0.10 ± 0.03</td>
<td>0.05 ± 0.02</td>
<td>0.00 ± 0.01</td>
</tr>
</tbody>
</table>

Figure S1. Mean voxel values for the clusters showing significant voxel-based morphometry differences between groups defined by diagnostic status at follow-up. Each bar shows the mean voxel value of the identified clusters. The error bars represent standard deviations. ADHD-FP: ADHD-full persisters; ADHD-PP: ADHD-partial persisters; ADHD-R: ADHD-remitters; L: left hemisphere; R: right hemisphere.
REFERENCES


Testing the differences in polygenic risk scores for ADHD between ADHD persistence and remission in a follow up of two European clinical samples

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1.1 Abstract

**Background:** There is little information on whether common genetic variants associated with an ADHD diagnosis also influence persistence and remission of ADHD. Here, we tested whether polygenic risk scores (PRS) for ADHD, derived from a recent large-scale ADHD genome-wide association study (GWAS), distinguish ADHD persisters from remitters in a clinical sample followed up from the International Multi-centre ADHD Gene project. **Methods:** Using an independent discovery sample (20,183 ADHD cases, 35,191 controls) PRS for ADHD were computed for 280 adolescents and young adults, who had childhood-onset ADHD, and for 169 unaffected individuals. Using data from a six-year follow-up, we examined the associations between ADHD-PRS and ADHD persistence/remission, as defined by parent-report, using both a categorical (DSM-IV based) and a dimensional approach. The impact of using self-report for diagnosis and controlling for known childhood clinical predictors of ADHD persistence was examined in sensitivity analyses. **Results:** PRS for ADHD were significantly higher in both persisters and remitters relative to controls, with limited impact of informant effects (i.e., parent or self-report). However, no significant associations between PRS for ADHD and ADHD persistence were observed in either the categorical or dimensional analyses. Results remained non-significant after controlling for childhood predictors of ADHD outcomes. **Conclusions:** Currently available genome-wide data on ADHD appear to capture common genetic determinants of the risk to obtain an ADHD diagnosis at any point in life, regardless of later ADHD status. Future studies will need to confirm this observation using increased sample sizes and explore compensatory mechanisms that may contribute to ADHD remission.
1.2 Introduction
Attention-deficit/hyperactivity disorder (ADHD) is a highly heritable condition (Faraone & Larsson, 2018). While its onset is often in childhood, ADHD persists into late adolescence and adulthood in most individuals with a childhood diagnosis (Cheung et al., 2015; Faraone, Biederman, & Mick, 2006; Langley et al., 2010; van Lieshout, Luman, Buitelaar, Rommelse, & Oosterlaan, 2013). When persisting into adulthood, ADHD has a high adverse impact on an individual’s life, leading to significantly worse educational, economic, health, and social outcomes (Barkley & Fischer, 2010; Cortese et al., 2013; Dalsgaard, Ostergaard, Leckman, Mortensen, & Pedersen, 2015; Fischer, Barkley, Smallish, & Fletcher, 2007; Klein et al., 2012). Finding early predictors of persistence of ADHD, therefore, is a priority.

Molecular genetic research has started to identify genetic risk factors for ADHD. The latest case-control genome-wide association study (GWAS), with more than 20,000 ADHD cases and 35,000 controls, found 12 genome-wide significant loci associated with ADHD (Demontis et al., in press). Building on these results, genetic studies now have the potential to inform on the risk factors for long-term outcomes of ADHD. However, no study to date has examined whether genetic risk factors for ADHD can predict the persistence and remission of a childhood ADHD diagnosis later in life.

Clinical features, cognitive and imaging data have been studied as potential predictors of long-term ADHD persistence. Previous studies have implicated severity of childhood ADHD symptoms, psychiatric comorbidities, family history of ADHD, atypical cortical maturation, lower IQ and, although not consistently, some other cognitive functions as potential contributors (Caye et al., 2016; Cheung et al., 2015; Larsson, Dilshad, Lichtenstein, & Barker, 2011; Shaw et al., 2013; Sjowall, Bohlin, Rydell, & Thorell, 2017). Follow-up studies further suggest that cognitive and neural impairment profiles differentiate ADHD remitters from persisters in adolescence and adulthood (Cheung et al., 2016; Halperin, Trampush, Miller,
Altogether, such findings support the hypothesis that distinct biological mechanisms underlie the different trajectories of ADHD.

To further investigate whether ADHD persistence and remission represent distinct biological subtypes, it would be informative to study the genetic determinants associated with these ADHD outcomes. Similar to other psychiatric disorders, ADHD is highly polygenic, in that disease risk is a consequence of several genetic factors of limited effect (Sullivan, Daly, & O'Donovan, 2012). Additive effects of all common genetic variants examined in genome-wide analyses have been found to explain a substantial proportion of its phenotypic variance (22%) (Demontis et al., in press). The complex genetic liability for psychiatric disorders can be quantified with polygenic risk scores (PRS), calculated as the sum of the risk alleles that contribute to the disorder weighted by their individual effects in genome-wide investigations (Wray et al., 2014). Applying this approach to a general-population sample, Riglin et al. (2016) obtained initial evidence that polygenic analyses may inform on the developmental changes of ADHD symptoms: individuals showing persisting ADHD traits from childhood to adolescence were characterised by the highest PRS for ADHD, while those with consistently low ADHD traits and those with high ADHD traits only early in childhood showed low polygenic risk for ADHD (Riglin et al., 2016).

However, such analyses have thus far only been performed defining ADHD outcome by the persistence/remission of ADHD symptoms (Riglin et al., 2016), rather than a combination of symptoms and functional impairments, limiting the generalisability of findings to clinical population samples with clinically diagnosed ADHD. To date, no data are available on the ADHD polygenic load in ADHD remitters and in individuals with persistent diagnosed ADHD. Therefore, the question remains whether the biological underpinnings of ADHD persistence
would also differ from those underlying its remission in clinical samples. Furthermore, the earlier PRS for ADHD were based on association results of the first GWAS from the Psychiatric Genomics Consortium (including 5,621 cases and 13,589 controls (Neale et al., 2010)), which was underpowered. Data from the recently expanded dataset in the latest ADHD-GWAS are now available (Demontis et al., in press) and, being more powerful, provide more accurate estimates of the effect sizes for PRS calculations than previously possible.

We now aim to examine the association of PRS derived from the latest and largest GWAS on ADHD (Demontis et al., in press) with ADHD remission and persistence, in a 6-year follow-up study of children and adolescents with diagnosed childhood DSM-IV ADHD, from the UK and Dutch subsamples of the International Multi-centre ADHD Genetics (IMAGE) project (Chen et al., 2008; Kuntsi et al., 2010; Rommelse et al., 2008). First, using a categorical approach, we contrast ADHD-PRS in ADHD persisters and remitters (i.e., defined based on symptomatic presentation and functional impairment, following DSM-IV criteria for ADHD); we further compare these two ADHD groups to non-ADHD controls. Second, using a dimensional approach, we investigate whether ADHD-PRS are associated with 1) the change in ADHD symptom scores from childhood to the 6-year follow-up, and 2) the number of persistent ADHD symptoms at follow-up. Third, as parent- and self-ratings of ADHD symptoms and impairment show limited agreement in their associations with objective – cognitive and neurophysiological – measures of ADHD remission and persistence (Du Rietz et al., 2016), we test ADHD-PRS for association with the persistence/remission of ADHD based on self- as well as parent-report. Fourth, we explore whether the polygenic contribution to ADHD persistence/remission was influenced by controlling for childhood clinical characteristics that have previously been linked to ADHD persistence, including symptom severity, having received pharmacological treatment for ADHD, and the presence of comorbid emotional and conduct problems in childhood (Caye et al., 2016).
1.3 Methods and Materials

1.3.1 Sample
Genotype array data for 294 participants with childhood DSM-IV combined-type ADHD and 175 unaffected individuals were available from two European cohorts: the UK (London site) and Dutch (Amsterdam and Nijmegen sites) subsamples of the IMAGE project (Chen et al., 2008; Kuntsi et al., 2010; Rommelse et al., 2008). Participants of the ADHD subsample were followed up on average 6.2 years (SD = 1.1) after initial assessments as part of two longitudinal studies in the UK and the Netherlands (Cheung et al., 2015; von Rhein et al., 2015). The unaffected subsample included control participants from the Dutch cohort (n=92) (Rommelse et al., 2008) and pseudo-control individuals from previous genome-wide association studies that included both cohorts (n=83) (Demontis et al., in press). Pseudo-controls were created using the non-transmitted alleles from the two parents of 83 of the included participants with childhood ADHD as described elsewhere (Demontis et al., in press; Neale et al., 2010). Details on recruitment and initial assessment procedures can be found in Appendix D. At follow-up, following exclusions due to missing clinical information and quality control of genotype data (described in section “Genotyping and quality control”), the final sample included 280 participants with childhood ADHD and 173 unaffected individuals. The investigation was carried out in accordance with the latest version of the Declaration of Helsinki. Study procedures were reviewed by local appropriate ethical committees, and informed consent of the participants was obtained after the nature of the procedures had been fully explained.

1.3.2 ADHD assessment
At follow-up, presence or absence of the nine-item inattention and nine-item hyperactivity-impulsivity DSM-IV ADHD symptoms were assessed using the Diagnostic Interview for ADHD in adults (DIVA) (Kooij & Francken, 2007) for the UK participants and the Schedule for Affective Disorders and Schizophrenia for School-Age Children –Present and Lifetime Version (KSADS-PL; (Kaufman et al., 1997)) for the Dutch participants. Absence or presence of impairment was...
assessed with the Barkley’s Functional Impairment Scale (Barkley & Murphy, 2006) for the UK participants and the KSADS-PL impairment items for the Dutch participants. Interviews were conducted by trained researchers, separately with participants and parents. For participants using medication, ratings were done regarding functioning off medication.

1.1.1.1. ADHD symptom change and ADHD symptom scores at follow-up

For the participants with childhood ADHD (n=280), we defined two quantitative measures of ADHD persistence: 1) the ADHD symptom change over time calculated as a difference score (symptom count at baseline – symptom count at follow-up) and 2) the ADHD symptoms score calculated as the ADHD symptom count at follow-up (Table 5.1). The total ADHD symptom count was obtained summing the scores on the hyperactivity-impulsivity and DSM-IV symptoms subscales from the parent interview.

Table 0.1. Characteristics of the childhood-ADHD group at baseline and follow-up assessments

<table>
<thead>
<tr>
<th></th>
<th>n=280</th>
<th>mean</th>
<th>SD</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td>11.4</td>
<td>2.7</td>
<td>5.4–18.0</td>
</tr>
<tr>
<td>Total ADHD symptoms&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>16.3</td>
<td>1.6</td>
<td>10–18</td>
</tr>
<tr>
<td><strong>Follow-up:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td>17.7</td>
<td>2.8</td>
<td>10.8–25.2</td>
</tr>
<tr>
<td>Total ADHD symptoms&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>11.0</td>
<td>4.5</td>
<td>0–18</td>
</tr>
<tr>
<td>ADHD symptom change&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>5.3</td>
<td>4.6</td>
<td>-8–18</td>
</tr>
</tbody>
</table>

ADHD: Attention Deficit/Hyperactivity Disorder. <sup>a</sup>Refers to the parent ratings of total ADHD symptom count at the clinical interview. <sup>b</sup>Refers to the change in total ADHD symptoms over time calculated by subtracting the symptom count at follow-up from the count at baseline obtained from parent interview.

1.1.1.2. ADHD diagnostic status at follow-up

Participants with a combined count of ≥6 inattention or hyperactivity-impulsivity symptoms and scored positive on two or more areas of impairment were classified as “affected” at follow-up. Details on the persistence rates based on parent-report for the UK and Dutch subsamples
have been reported previously (Cheung et al., 2015; van Lieshout et al., 2013). Here, of those with a childhood-ADHD diagnosis (n=280), 179 continued to meet DSM-IV criteria for ADHD at follow-up and were classified as ADHD persisters, while 67 were classified as ADHD remitters based on parent-report (Table 5.2). Two control participants met ADHD criteria at the diagnostic interview and were excluded from all analyses. Thirty-four individuals with childhood ADHD and two controls met ADHD criteria for impairment, but not for symptoms; these participants were excluded from the categorical analyses based on parent-report as their diagnostic status could not be ascertained. For sensitivity analysis, we further divided those with a childhood-ADHD diagnosis based on self-report. When using self-report, among the childhood-ADHD participants (n=280), 99 were classified as ADHD persisters and 108 were classified as ADHD remitters (Table 5.2). Fourteen control participants and 20 participants with ADHD had missing self-rated symptoms/impairment at follow-up, while one control and 49 ADHD probands met ADHD criteria for clinical impairment, but not for symptoms. These participants were excluded from the self-report-based categorical analyses.
Table 0.2. Sample characteristics of participants at follow-up

<table>
<thead>
<tr>
<th></th>
<th>Diagnostic groups based on parent-report</th>
<th>Diagnostic groups based on self-report</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADHD</td>
<td>ADHD</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>remitters</td>
</tr>
<tr>
<td>(n=169)</td>
<td>(n=67)</td>
<td>(n=179)</td>
</tr>
<tr>
<td>Males, n (%)</td>
<td>118 (69%)</td>
<td>53 (79%)</td>
</tr>
<tr>
<td>Age (years), mean ± SD</td>
<td>17.4 ± 3.5</td>
<td>17.9 ± 2.8</td>
</tr>
<tr>
<td>Total ADHD symptoms^a</td>
<td>0.3 ± 1.1</td>
<td>6.9 ± 4.7</td>
</tr>
</tbody>
</table>

^aRefers to the parent- and self-ratings of total ADHD symptom count at the clinical interview at follow-up; scores were not available for the 83 pseudo-controls.
1.3.3 Childhood measures
From the initial assessments, we used ADHD symptom scores (symptom count), use of ADHD medications at any point prior to study participation, and parent-rated scores of emotional and conduct problems measured with the Strengths and Difficulties Questionnaire (Goodman, 1997) at initial assessments.

1.3.4 Genotyping and quality control
All UK participants (n=122) and 40 of the Dutch participants were genotyped using the Perlegen 600K platform (Perlegen Sciences, Santa Clara, USA). The remaining Dutch participants were genotyped on the Illumina Infinium PsychArray-24v1.1 (Illumina, San Diego, USA). Quality control and imputation were performed using the PGC pipeline "Ricopili" as described in (Schizophrenia Working Group, 2014). The genotypes on the Perlegen (as already described in Demontis et al., in press) and Illumina arrays were processed separately. The imputation reference panel consisted of 2,504 phased haplotypes from the 1000 Genomes Project, phase 3 (The Genomes Project, 2015). After imputation, we excluded individuals with genotyping rate below 1% and single-nucleotide polymorphisms (SNPs) with minor allele frequency below 5%, genotypic rate below 1%, out of Hardy Weinberg equilibrium (p<1x10^-6) and of poor imputation quality (INFO score <0.8). In addition, related individuals, identified based on Identity-By-Descent above 0.125, and individuals with mismatch between recorded and genotypic sex were removed from analyses. After completing quality control procedures separately in each subsample, we merged the two datasets using the overlapping markers in the two arrays and repeated the quality control on the combined dataset. The resulting dataset included 2,267,838 variants and 459 participants available for analyses.

1.3.5 Polygenic risk scores
To calculate PRS for each individual in our sample, we used as the discovery dataset the latest Psychiatric Genomics Consortium European ADHD GWAS meta-analysis (Demontis et al., in
press) – excluding all participants of the IMAGE project and those with non-European genetic ancestry, resulting in 18,399 cases and 33,494 controls with European ancestry. Polygenic scores were calculated using PRSice (Euesden, Lewis, & O'Reilly, 2015). An $r^2 \geq 0.1$ (250-kb window) was used for clumping to remove SNPs in linkage disequilibrium. Only SNPs overlapping between the discovery and the target sample were included in the clumping procedure. After clumping, we included 60,491 variants in the PRS analyses. PRS were calculated for eight subsets of SNPs, selected by their association p-value in the discovery sample, with thresholds ranging from 0.01 and 1.0. Table 5.3 displays the number of independent genetic variants included in PRS calculation at each threshold.

<table>
<thead>
<tr>
<th>p-value threshold (pT)</th>
<th>SNPs (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p &lt; 0.01</td>
<td>3440</td>
</tr>
<tr>
<td>p &lt; 0.05</td>
<td>10157</td>
</tr>
<tr>
<td>p &lt; 0.1</td>
<td>16204</td>
</tr>
<tr>
<td>p &lt; 0.2</td>
<td>25485</td>
</tr>
<tr>
<td>p &lt; 0.3</td>
<td>32965</td>
</tr>
<tr>
<td>p &lt; 0.4</td>
<td>39285</td>
</tr>
<tr>
<td>p &lt; 0.5</td>
<td>44587</td>
</tr>
<tr>
<td>p &lt; 1</td>
<td>60491</td>
</tr>
</tbody>
</table>

### 1.3.6 Statistical analyses

A preliminary analysis first aimed to confirm the differences in ADHD-PRS between childhood ADHD cases and controls that were previously reported in a sample that also included our participants (Demontis et al., in press). Second, following the study aims, we tested the association of ADHD-PRS with the persistence/remission status among individuals with
childhood ADHD, as well as with case-control status by comparing ADHD remitters versus controls and ADHD persisters versus controls. Case-control comparisons were completed to ascertain whether the genetic signal underlying ADHD can be detected in a sample of modest sample size such as that included in our study, and for comparability of the variance explained across analyses. All pairwise comparisons were performed using logistic regressions. Among those with childhood ADHD, PRS for ADHD were then tested for association with the ADHD symptom change over time and with ADHD symptom count at follow-up. ADHD symptom change scores were log-transformed to normality before being entered in linear regressions and ADHD symptom count at follow-up were modelled using Poisson regressions.

We included the first ten ancestrally informative principal components, genotyping array, site of follow-up assessment, and age at follow-up as covariates in all analyses. The amount of variance explained by ADHD-PRS was calculated as the difference of Nagelkerke’s pseudo-$R^2$ in the full model as compared to the null model, which included all covariates but not the PRS. For each analysis, we estimated and analysed PRS at eight different levels of significance in the discovery sample, consistent with prior studies (Martin, Hamshere, Stergiakouli, O’Donovan, & Thapar, 2014; Stergiakouli et al., 2015). To correct for multiple testing, we estimated the equivalent number of effective tests using the correlation matrix of the participants’ PRS at the eight $p$-value thresholds (http://gump.qimr.edu.au/general/daleN/matSpD/) and performed Bonferroni correction on the sum of effective tests. The effective number of independent variables was 2.8, with a total of 5 tests in the main analysis, and the multiple-testing adjusted $p$-value was determined to be 0.0036.

To better visualize the effect of PRS on ADHD outcomes, we estimated the association of the most associated ADHD-PRS in each regression model with the risk of ADHD persistence dividing the selected outcome and the sample into quintiles by PRS.
To test whether the associations of ADHD-PRS with ADHD persistence/remission remain the same controlling for potential childhood clinical predictors, we first tested the associations of these factors with the ADHD outcomes. Among the childhood measures at initial assessments, ADHD symptom scores and parent-reports of conduct problems, but not use of ADHD medications at any point prior to initial assessments or emotional problems, were significant covariates and were therefore retained in the analyses. Accordingly, we repeated the regression models examining ADHD symptom change and persistence/remission status controlling for ADHD symptom scores and conduct problems at initial assessments.

1.4 Results

1.4.1 Preliminary analysis: ADHD-PRS in all childhood-ADHD probands versus controls
Analyses confirmed the previous finding (Demontis et al., in press) of significantly increased ADHD-PRS in individuals with childhood ADHD relative to controls, with the most predictive PRS explaining 6.2% of the variance (pseudo-$R^2$; $p<0.001$) (Figure 5.1).
**Figure 0.1.** Proportion of variance explained by ADHD-PRS (A) and quantiles of polygenic risk scores plotted against effects of ADHD-PRS on the risk of ADHD (B) for the case-control status based on baseline assessments.

(A) The bars represent the variance explained by each ADHD-PRS calculated for eight subsets of genetic variants at different p-value thresholds in the discovery ADHD case-control GWAS. The number on top of each bar represents the p-value of the association of the given ADHD-PRS with the
outcome. (B) Odds Ratios (OR) for the risk of ADHD and 95% CI for the quantile groups (bars) are represented for the case-control comparison. The threshold for selecting ADHD-associated alleles was pT=0.1.
1.4.2 Do ADHD-PRS differentiate ADHD persisters, ADHD remitters, and controls using diagnostic categories defined by parent-report at follow-up?

PRS that included genetic variants at the p-value threshold (pT) of 0.05 or greater in the discovery GWAS significantly distinguished ADHD persisters from controls (Figure 5.2). Optimal discrimination was achieved with the inclusion of variants associated with ADHD at pT=0.5, which explained 5.9% of the variance (pseudo-$R^2$; p<0.001). The probability of having an ADHD diagnosis at follow-up was greater with the increase in ADHD-PRS (Figure S5.1, Appendix D).

ADHD-PRS at all p-value thresholds except pT=0.01 significantly differentiated between the ADHD remitters and controls (Figure 5.2). The polygenic score including genetic variants associated with ADHD with a pT=0.1 in the discovery GWAS explained the greatest proportion (6.5%) of the variance in the remitter-control status (pseudo-$R^2$; p<0.001), with higher ADHD-PRS being associated with greater risk for ADHD (Figure S5.1, Appendix D).

Finally, none of the examined subsets of genetic variants associated with ADHD in the discovery GWAS distinguished ADHD persisters and remitters as defined by parent-report (Figure 5.2). The quantile plot for this non-significant association is depicted in Figure S5.1 (Appendix D).
Figure 0.2. Proportion of variance explained by ADHD-PRS for the persistence-control, remission-control and persistence-remission status at follow-up based on parent-report.

The bars represent the variance explained by each PRS calculated for eight subsets of genetic variants at different p-value thresholds in the latest Psychiatric Genomics Consortium ADHD meta-analysis. The number on top of each bar represents the p-value of the association of the given ADHD-PRS with the outcome.
1.4.3 Are ADHD-PRS associated with ADHD symptom change and number of symptoms at follow-up in those with childhood ADHD?

Among individuals with childhood ADHD, the association of the ADHD-PRS with ADHD symptom change over time and ADHD symptom scores at follow-up did not reach our threshold for statistical significance (Figure 5.3). Quantile plots of these non-significant associations, generated for exploratory purposes, revealed that ADHD-PRS tended to decrease with greater symptom improvement and with lower severity at follow-up (Figure S5.2, Appendix D).

1.4.4 Sensitivity analyses: impact of using self-report for diagnosis and controlling for known childhood predictors of ADHD outcomes

ADHD-PRS with a pT=0.05 or greater significantly distinguished ADHD persisters from controls defined by self-report (Figure 5.4). Optimal discrimination was achieved when including variants at pT=0.5, which explained 6.9% of the variance (pseudo-R²; p<0.001), and increased with increased risk for persistent ADHD (Figure S5.3, Appendix D). No ADHD-PRS revealed associations strong enough to reach our threshold for statistical significance in the persisters-remitters or the remitters-controls comparisons, when the diagnosis was based on self-report (Figure 5.4; Figure S5.3, Appendix D). When controlling for ADHD symptom scores and conduct problems at initial assessments, the association of ADHD-PRS with the persistence/remission remained non-significant in both continuous (ADHD symptom scores) and categorical (ADHD diagnosis) analyses (Figure 5.5).
Figure 0.3. Proportion of variance explained by ADHD-PRS for the ADHD symptom change and ADHD symptoms severity at follow up based on parent report (left and right panel, respectively) in the individuals with childhood ADHD.

The bars represent the variance explained by each PRS calculated for 8 subsets of genetic variants at different p-value thresholds in the latest Psychiatric Genomics Consortium ADHD meta-analysis. The number on top of each bar represents the p-value of the association of the given ADHD-PRS with the outcome.
Figure 0.4. Proportion of variance explained by ADHD-PRS for the persistence-control, remission-control and persistence-remission status at follow-up based on self-report.
The bars represent the variance explained by each PRS calculated for eight subsets of genetic variants at different p-value thresholds in the latest Psychiatric Genomics Consortium ADHD meta-analysis. The number on top of each bar represents the p-value of the association of the given ADHD-PRS with the outcome.

A. ADHD symptom change (parent report)

B. ADHD-Persisters vs. ADHD-Remitters (parent report)

C. ADHD-Persisters vs. ADHD-Remitters (self report)
Figure 0.5. Proportion of variance explained by ADHD-PRS for the ADHD symptom change (A), ADHD persistence-remission defined by parent report (B), and ADHD persistence-remission based on self-report (C), controlling for known childhood predictors of ADHD outcome.

The bars represent the variance explained by each PRS calculated for eight subsets of genetic variants at different p-value thresholds in the latest Psychiatric Genomics Consortium ADHD meta-analysis. The number on top of each bar represents the p-value of the association of the given ADHD-PRS with the outcome when analyses were repeated controlling for ADHD symptoms, use of ADHD medications, and emotional and conduct problems at baseline.
1.5 Discussion

Using polygenic risk scores derived from the most recent ADHD GWAS meta-analysis (Demontis et al., in press), we examined whether biological mechanisms underlying ADHD also influence its developmental outcomes – persistence and remission – in a clinical sample. Analyses confirmed prior reports (Demontis et al., in press) that high polygenic risk for ADHD distinguishes childhood-ADHD probands and controls. Both ADHD persisters and remitters also differed from unaffected individuals when outcome was defined by parent-report and a similar pattern of results emerged when using self-report for the diagnostic procedure. Conversely, no ADHD polygenic risk score effects emerged on the persistence/remission of ADHD, and results did not change when controlling for known clinical childhood markers of long-term ADHD outcomes (Caye et al., 2016). These findings, if replicated in future studies with larger samples, tentatively suggest that the genetic variation associated with ADHD risk may not be the same as the genetic risk factors that contribute to the persistence or remission of the disorder.

Increased polygenic risk for ADHD was observed in all individuals with childhood ADHD, regardless of current diagnostic status, consistent with the findings of the latest ADHD GWAS, which also included our sample (Demontis et al., in press). Despite the small size of our samples, we additionally found that both ADHD persisters and remitters showed significantly higher polygenic risk for ADHD relative to controls, suggesting an overlap in genetic susceptibility to ADHD in both subsamples. In both these comparisons, the ADHD-PRS explained approximately 6% of the ADHD risk variance. It is worth noting that, such estimates of variance explained are similar to those observed in prior case-control analyses within the most recent ADHD GWAS (5.5%, (Demontis et al., in press)) and in other highly polygenic psychiatric disorders such as schizophrenia (6%, (Schizophrenia Working Group, 2014)). This indicates that current polygenic approaches, exploiting the power of the recently published ADHD
GWAS, have the ability to identify the polygenic load of ADHD even in relatively small samples.

Analyses did not detect a significant influence of PRS for ADHD on symptom change or on persistence/remission of ADHD as a diagnosis. Based on the findings of the only previous study which reported increased ADHD polygenic risk in individuals with persistent ADHD symptoms, but not in those with childhood-limited symptoms, in the general population (Riglin et al., 2016), these results were unexpected. Here, although we used a more powerful ADHD GWAS to estimate effects of common genetic variants, the non-significant polygenic effects on persistence vs. remission may be due to a small sample size. The discrepancy with the study by Riglin et al. (2016) may also come from the difference in the samples examined. In our clinical sample, high ADHD scores and relatively small symptom change characterised individuals with childhood-onset ADHD. The small clinical change may have limited the variance and therefore also our power to detect associations with ADHD-PRS compared to analyses on the full spectrum of symptoms in general-population samples. Additionally, while the polygenic effects in Riglin et al. (2016) were based on the persistence of ADHD traits only, we additionally applied a categorical approach using both ADHD symptoms and functional impairments to inform a clinical diagnosis of persistent/remittent ADHD.

Moreover, the GWAS currently available to derive PRS for ADHD included youths and adults with a diagnosis of ADHD at the time of the investigation, regardless of later outcomes. Therefore, it is possible that PRS for ADHD generated from such a heterogeneous sample capture the biological mechanisms underlying the liability to ADHD, but not necessarily those that contribute to its persistence. Alternatively, the PRS derived from the current ADHD-GWAS may include a mixture of risk alleles for the onset
and risk alleles for persistence of ADHD. In recent twin studies, it has been shown that genetic factors linked to ADHD onset may be independent of those that contribute to the symptom maintenance in late adolescence/young adulthood (Chang, Lichtenstein, Asherson, & Larsson, 2013; Pingault et al., 2015). This would also be in line with studies that implicated some, but not all, genes related to childhood ADHD in the genetics of adults with the disorder (for a review, see Franke et al., 2012).

Other factors might influence ADHD persistence. Evidence is accumulating that the functioning of certain neuro-cognitive processes typically impaired in individuals with ADHD may improve in ADHD remitters, and not in persisters, in follow-up studies (Biederman et al., 2009; Cheung et al., 2016; Francx et al., 2015; Halperin et al., 2008; Mattfeld et al., 2014; McAuley et al., 2014; Michelini et al., 2016; Pazvantoglu et al., 2012; Szekely, Sudre, Sharp, Leibenluft, & Shaw, 2017). Despite the observed high genetic liability to ADHD in both ADHD persisters and remitters in our study, the findings of possible brain and cognitive markers of ADHD remission suggest that compensatory mechanisms might occur in the individuals who remit from the disorder. Therefore, future studies should also aim to investigate whether other determinants, including environmental factors and gene-x-environment interactions, underlie the pathway to ADHD persistence.

Informant effects only marginally impacted the results of the polygenic prediction on ADHD outcomes. No associations between ADHD-PRS and persistence-remission status emerged when outcome was defined by self-report, similar to parent-report. The significant association of ADHD-PRS with the persistence-control status observed in the main analysis was confirmed when using self-report to define ADHD outcome. However, the observed increase in PRS for ADHD in ADHD remitters defined by parent-report did
not reach statistical significance when using self-report. These borderline results suggest that genetic data may be sensitive to the ADHD risk regardless of the informant available at the diagnostic assessment. We further note that, for self-report, PRS for ADHD explained approximately half of the variance explained by the polygenic scores for remission-control differences using parent report, despite the larger sample size in the remitters defined by self-report (n=111) than those defined by parent-report (n=67). A partial convergence between parent- and self-report is also suggested in the latest ADHD GWAS, where findings were replicated in participants with ADHD defined by self-report, although with only a moderate agreement between informants (Demontis et al., in press).

PRS for ADHD also did not show association with ADHD outcomes when controlling for symptom severity, use of medication, emotional and conduct problems at initial assessments, which emerged as significant markers of ADHD persistence in a recent meta-analysis (Caye et al., 2016). This finding is in line with the results of the study by Riglin et al. (2016), where polygenic effects on the persistence/remission of ADHD traits became non-significant when controlling for a neurodevelopmental index including social communication difficulties, cognitive abilities, and conduct problems. Findings from our clinical sample and those from a general population sample, therefore, converge in indicating that the predictive validity of polygenic risk scores is not yet sufficient, alone or in combination with other phenotypic characteristics, to identify individuals at high risk of enduring symptoms and impairments.

Beyond possible low statistical power in our relatively small sample, a limitation of this study is that we examined only participants diagnosed with ADHD combined subtype in childhood to reduce heterogeneity in the sample. Thus, future studies will need to clarify
whether our findings generalize to other presentations of ADHD. Furthermore, our sample included young adults as well as adolescents, who are still undergoing cortical maturation (Castellanos et al., 2002; Shaw et al., 2006) and may further change their clinical presentation in later ages. Although we controlled for age in all analyses, future follow-up assessments with participants having reached adulthood and when more ADHD participants may have remitted could clarify matters further. Finally, among the ADHD remitters, 17 (25%) individuals reported to have continued using ADHD medication at follow-up. Yet, ADHD persistence/remission in categorical analyses was based on symptoms and impairments ratings evaluated on medication-free periods, likely indicating that medication use did not impact our results. Further, remitter-control analysis repeated excluding these individuals revealed similar effect sizes.

In summary, in a follow up of individuals with childhood ADHD, high polygenic risk for ADHD did not emerge as a significant predictor of ADHD persistence or remission in either a categorical or a dimensional approach. Results remained unchanged when controlling for potential effects of childhood markers of ADHD persistence and revealed a partial agreement between informants (i.e., parent- and self-report). Currently available genome-wide data on ADHD are likely to reflect genetic risk of receiving an ADHD diagnosis at any point in life, regardless of the persistence/remission outcomes. Future studies will need to further study this observation using larger samples and explore whether rare genetic variants, environment and/or gene-x-environment interaction effects may explain the possible compensatory mechanisms leading to ADHD remission.
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neurophysiological impairments and ADHD symptoms consistent with a causal inference or due to familial confounds?


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Research report

The executive control network and symptomatic improvement in attention-deficit/hyperactivity disorder

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Abstract

Background: One neurodevelopmental theory hypothesizes remission of attention-deficit/hyperactivity disorder (ADHD) to result from improved prefrontal top-down control, while ADHD, independent of the current diagnosis, is characterized by stable non-cortical deficits (Halperin & Schulz, 2006). We tested this theory using resting state functional MRI (fMRI) data in a large sample of adolescents with remitting ADHD, persistent ADHD, and healthy controls.

Methods: Participants in this follow-up study were 100 healthy controls and 129 adolescents with ADHD combined type at baseline (mean age at baseline 11.8 years; at follow-up 17.5 years). Diagnostic information was collected twice and augmented with magnetic resonance imaging (MRI) scanning at follow-up. We used resting state functional connectivity (RSFC) of the executive control network to investigate whether improved prefrontal top-down control was related to a developmental decrease in ADHD symptoms. In addition,
we tested whether non-cortical RSFC, i.e., cerebellar and striatal RSFC, was aberrant in persistent and/or remittent ADHD compared to controls.

Results: Higher connectivity within frontal regions (anterior cingulate cortex) of the executive control network was related to decreases in ADHD symptoms. This association was driven by change in hyperactive/impulsive symptoms and not by change in inattention. Participants with remitting ADHD showed stronger RSFC than controls within this network, while persistent ADHD cases exhibited RSFC strengths intermediate to remittent ADHD cases and controls. Cerebellar and subcortical RSFC did not differ between participants with ADHD and controls.

Conclusions: In line with the neurodevelopmental theory, symptom recovery in ADHD was related to stronger integration of prefrontal regions in the executive control network. The pattern of RSFC strength across remittent ADHD, persistent ADHD, and healthy controls potentially reflects the presence of compensatory neural mechanisms that aid symptomatic remission.

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1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) has a variable clinical course. While 30–50% of children diagnosed with ADHD exhibit symptomatic improvement throughout development (Biederman, Mick, & Faraone, 2000), others show persistent ADHD behavior into adulthood (Faraone, Biederman, & Mick, 2006). Adult ADHD has a substantial impact on the public health system as it has been associated with impairments in multiple domains including family functioning, work, and leisure time (Biederman et al., 2006; Kessler et al., 2006). Few behavioral, environmental, and neurocognitive factors were found to predict clinical outcome (Biederman, Petty, Clarke, Lomedico, & Faraone, 2011; Kessler et al., 2005; van Lieshout, Luman, Buitelaar, Rommelse, & Oosterlaan, 2013). However, using brain measures we might be able to identify the mechanisms underlying remittent and persistent ADHD trajectories.

According to the neurodevelopmental theory formulated by Halperin and Schulz (2006) ADHD is characterized by relatively stable non-cortical dysfunctions. This theory is primarily based on evidence from cognitive investigations showing that ADHD is related to deficits in so-called lower cognitive mechanisms, as demonstrated by increased variability of reaction times in more automatic and less effortful tasks (Bedard, Trampush, Newcorn, & Halperin, 2010; Halperin, Trampush, Miller, Marks, & Newcorn, 2008). Recent magnetic resonance imaging (MRI) studies reporting on the cerebellum and subcortical structures including caudate, nucleus accumbens, and putamen, have provided additional neurobiological support for the hypothesized non-cortical dysfunctions in ADHD (Frodl & Skokauskas, 2012; Hart, Radua, Mataix-Cols, & Rubia, 2012; Hart, Radua, Nakao, Mataix-Cols, & Rubia, 2013; Nakao, Radua, Rubia, & Mataix-Cols, 2011; Stoodley, 2014; Valera, Faraone, Murray, & Seidman, 2007).

Importantly, stable non-cortical dysfunctions are not only hypothesized in persistent, but also in symptomatically improved (i.e., remittent) ADHD cases. Indeed, a recent functional MRI (fMRI) study in adults with childhood ADHD reported that reaction time task performance, which relies on non-cortical regions, was not related to symptom recovery (Clerkin et al., 2013). Furthermore, decreased activation in the thalamus was found independent of current diagnosis. Additional support was provided by structural MRI studies that reported non-progressive reductions in striatal surface area in ADHD (Shaw et al., 2014) and cerebellar volume reductions in remittent ADHD (Mackie et al., 2007), indicating persistent non-cortical deficits. However, also evidence has been reported that is in conflict with the developmental theory and points to absence of structural basal ganglia deficits in adults with ADHD (Frodl & Skokauskas, 2012; Nakao et al., 2011) and findings indicating volumetric normalization with aging (Castellanos et al., 2002).

Next to non-cortical dysfunctions, cortical dysfunctions have also been related to ADHD pathology. One of the most replicated findings is abnormal structure and functioning of the prefrontal cortex (PFC) (Cortese et al., 2012; Hart et al., 2012). Within the neurodevelopmental theory, the development of PFC has been hypothesized to parallel improvements in ADHD symptoms. Such PFC compensation would be reflected in improved performance on effortful executive functioning tasks that depend on PFC functioning. Indeed, better performance on working memory, inhibition, and, sustained attention tasks has been reported in remittent compared to persistent ADHD (Bedard et al., 2010; Fischer, Barkley, Smallish, & Fletcher, 2005; Halperin et al., 2008). However, other studies were unable to document improvements in performance of executive function tasks (Biederman et al., 2009; Mick et al., 2011; van Lieshout et al., 2013).

Measures of brain structure and function might augment our insight into the mechanisms underlying remission of ADHD by revealing normalization of brain function or by revealing compensatory changes (Fassbender & Schweitzer, 2006; Giedd & Rapoport, 2010; Rubia, 2002; Shaw, Gogtay, & Rapoport, 2010). Accordingly, previous studies have linked diagnostic outcome to the status of the PFC. ADHD outcome has been associated with the developmental trajectories of...
the medial PFC and cingulate extending to the precuneus (Shaw et al., 2013). In addition, stronger functional integration between the thalamus and prefrontal areas, as measured using fMRI, paralleled symptom recovery (Clerkin et al., 2013). However, this report did not find support for the prediction that increased prefrontal activation was related to ADHD recovery. Finally, a recent study demonstrated normalization of resting state functional connectivity (RSFC) between the posterior cingulate and medial PFC regions in remitted ADHD (Mattfeld et al., 2014).

The aim of the present study was to directly test the theory that prefrontal cortical functioning provides a mechanism aiding ADHD remission. To investigate prefrontal functioning we assessed the connectivity of a cognitive control network based on resting state fMRI (rfMRI) data instead of using a task-paradigm that is limited to certain cognitive functions (e.g., working memory). Specifically, we investigated whether symptom remission in ADHD, i.e., a decrease in symptoms throughout development, was related to prefrontal connectivity of the executive control network. In addition, we evaluated the presence of aberrant non-cortical connectivity in cerebellar and striatal networks in both persistent and remitted ADHD cases, compared to healthy controls.

2. Methods

2.1. Participants

All participants were part of a prospective longitudinal study, the International Multicenter ADHD Genetics (IMAGE) study (as described previously in Muller et al., 2011a, 2011b; Nijmeijer et al., 2009; Rommelse et al., 2008). Participants with ADHD combined type were recruited from outpatient psychiatric or pediatric clinics, ADHD diagnoses were reassessed using the Parental Account of Children’s Symptoms Questionnaire (K-SADS; Kaufman et al., 1997) and Conners’ rating scales. Control families were recruited from schools and did not meet criteria for ADHD, neither did their first-degree relatives. Further inclusion criteria for both groups were an IQ > 70, European Caucasian descent, and no diagnosis of autism, epilepsy, general learning difficulties, brain disorders, or known genetic disorders (such as Fragile X or Down syndrome). IQ was estimated based on the WISC or WAI$-	ext{III Vocabular}$, SIMILARITIES, PICTURE COMPLE$-$TION, and BLOCK DESIGN subtests at baseline. Diagnostic, neurocognitive, and genetic data were collected at the University of Amsterdam and Radboud University Medical Centre Nijmegen.

All participants were invited for follow-up after 5 years (mean follow-up = 5.9 years, SD = 4.9) during which the protocol was complemented with the acquisition of MRI brain scans, including a rfMRI scan (NeuroMAGE study: as described in von Rhein et al., 2014)). Follow-up rates were 75.6% for ADHD families and 74.9% for control families. ADHD diagnoses were re-assessed using the Dutch translation of the Schedule for Affective Disorders and Schizophrenia for School-Age Children [K-SADS (Kaufman et al., 1997)] and Conners’ rating scales. Comorbidity with oppositional defiant disorder (ODD) and conduct disorder (CD) were assessed using the K-SADS. Initially only the screening interview was administered, thereafter participants with elevated scores on any of the screen items were also administered the full section. For participants using medication, the participants’ functioning was rated when off medication. Duration of medication use was registered based on pharmacy reports. All participants gave informed consent and the study was approved by local ethical committees.

Longitudinal diagnostic information and a rfMRI scan were available for 274 participants. For the current analyses we excluded participants with a rfMRI scan of bad quality after visual inspection (n = 18, of which 1 healthy control) and the 5% participants with highest head-motion (n = 27, of which 2 healthy controls), as determined by frame-wise displacement [cut-off at .73 root mean squared head position change (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012)]. We included 129 participants with ADHD combined type at baseline and 100 healthy controls in our final analyses. For both groups, there were no differences between the participants included in the current analysis and the complete sample on measures of ADHD severity, age, and gender (p > .05).

2.2. Longitudinal ADHD assessment

As all participants were assessed at both measurements using a parent-rated Conners’ questionnaire (Conners’ Parent Rating Scale — Revised: Long version — CPRS-R:L; Conners, Sitarenios, Parker, & Epstein, 1998), we used this questionnaire to determine ADHD symptoms at each time point. Two Conners’ scales were used: DSM-Inattentive behavior (scale L of the CPRS-R:L) and DSM-Hyperactive/Impulsive behavior (scale M of the CPRS-R:L) scales. At follow-up, scores on the Conners’ questionnaires were highly correlated with symptoms counted based on the Schedule for Affective Disorders and Schizophrenia for School-Age Children — Present and Lifetime Version [K-SADS (Kaufman et al., 1997)] (for inattention r = .77; for hyperactivity/impulsivity r = .76).

2.3. Imaging and preprocessing

MRI data were acquired at follow-up at two locations (VU University Amsterdam and Radboudumc in Nijmegen), with 1.5T scanners from Siemens (MAGNETOM Sonata and Avanto). Both scanners were equipped with 8-channel phased-array head coils. For each participant a T1-weighted anatomical scan was collected (TR 2730 msec, TE 2.95 msec, T1 1000 msec, voxel size 1 × 1 × 1 mm, flip angle 7°, matrix size 256 × 256, FOV 256 mm, 176 slices). Nine minutes of rfMRI data was acquired using a gradient-echo echo-planar imaging (GE-EPI) sequence (TR 1960 msec, TE 40 msec, flip angle 80°, matrix size 64 × 64, in-plane resolution 3.5 mm, FOV 224 mm, 35 axial slices, slice thickness/gap 3.0 mm/0.5 mm, 265 volumes). Participants were instructed to relax while keeping their eyes open.

rfMRI data were preprocessed using the FMRIB Software Library (FSL; http://www.fmrib.ox.ac.uk/fsl). We applied standard preprocessing including removal of the first five volumes to allow for signal equilibration, primary head movement correction via realignment to the middle volume (FSL-MCFLIRT), grand mean scaling, and spatial smoothing.
using a 6 mm FWHM Gaussian kernel. Subsequently, to correct for secondary effects of head motion we used an advanced ICA-based strategy for automatic detection and removal of motion-related artifacts (ICA-AROMA; Pruim et al., under review). ICA-AROMA automatically selects the motion-related components as identified by independent component analysis (ICA) (FSL MELODIC). After removing these components identified as motion-related artifacts (using fsl_regfilt), we additionally removed signal from white matter and cerebrospinal fluid using nuisance regression, and applied a high-pass filter (0.01 Hz). Functional images were co-registered to the anatomical image using boundary-based linear registration (Greve & Fischl, 2009). Anatomical images were non-linearly transformed to MNI152 space (FSL-FNIRT). Concatenation of both transformations was used to bring participant-level rfMRI data to MNI152-standard space.

2.4. Resting state network characteristics

Over the last decades the focus of neuroimaging research has moved from investigating activity in individual regions to the study of large-scale distributed network activity. Brain regions showing spontaneous fluctuations in activation patterns over time are delineated as a functional network. A data-driven approach to delineate these resting state networks is ICA (Beckmann, DeLuca, Devlin, & Smith, 2005). The estimated components or networks are spatially highly consistent over subjects (Damoiseaux et al., 2006). Furthermore, although measured during rest, these networks are related to activation patterns measured during tasks (Laird et al., 2011; S. M. Smith et al., 2009).

Of note, although data-driven approaches can be used to delineate resting state networks, the field of resting state networks is not without ambiguity. Networks do not map directly on behavior and previously defined ontology. As a result, variation in definitions of networks is induced. One example can be found in the subdivision of one network into various sub-systems.

A network that is of particular interest for ADHD is a network that has been related to executive control and salience processing (Seeley et al., 2007). Several cognition paradigms that are affected in ADHD, e.g., Stroop and go/no-go tasks, show similar brain activation patterns as this executive control network (Beckmann et al., 2005; Cortese et al., 2012; Hart et al., 2012). Areas of activation in this network include medial-frontal areas (including the anterior cingulate and paracingulate cortex, and superior, middle, and ventrolateral prefrontal cortices; Beckmann et al., 2005), and a part of the thalamus which connects to the PFC (Fair et al., 2010; Johansen-Berg et al., 2005), Fig. 1.

2.5. Selection and extraction of resting state networks

Based on our hypothesis that symptomatic improvement is accompanied by increased prefrontal functioning, we focused on the executive control resting state network (RSN) for further analysis. This is one of the 20 well-identified RSNs that were created using ICA (S. M. Smith et al., 2009). The pre-defined executive control RSN was spatially regressed against the individual subjects’ fMRI data, identifying the subject-specific time courses. The temporal relationship between the RSN time course and each voxel’s time course is called ‘resting-state functional connectivity’ (RSFC).

Since we hypothesized non-cortical RSFC to be related to ADHD, independent of remittent or persistent status, we also assessed RSFC of cerebellar and subcortical resting state networks (Fig. 1). For the cerebellar network the same procedure was followed as for the executive control network, i.e., this is

![Fig. 1 – Executive control RSN and cerebellar RSN created based on spatial maps of 20 well-defined RSNs (S. M. Smith et al., 2009). Subcortical RSNs created based on individual subcortical seeds of the nucleus accumbens, caudate, and putamen.](image-url)
also a RSN from the 20 well-identified RSNs. To specifically investigate the connectivity of the subcortical regions with the rest of the brain, we created subcortical RSNs. First, we created subject-specific masks of the nucleus accumbens, caudate nucleus, and putamen. Each subcortical region of interest (ROI) was automatically segmented using the individual structural scan in native space [FSL-FIRST, (Patenaude, Smith, Kennedy, & Jenkinson, 2013)], subsequently, the ROIs were transformed into functional space and binarized. Next, for each mask the eigenvariate of the time courses was extracted using singular value decomposition. The first eigenvariate is a summary of the time courses in the ROI (similar to the mean), however, it is less biased when heterogeneity between voxels in the ROI is present. Finally, the eigenvariate of all ROIs were entered in one regression model. By using this approach we obtained unique whole-brain functional connectivity maps for each subcortical seed.

2.6. Statistical analyses

The associations between rfMRI and behavioral data were tested by means of two statistical models: (1) a dimensional model relating change in ADHD symptoms to inter-individual differences in RSFC, and (2) a categorical model directly comparing RSFC between typical groups of remittent ADHD, persistent ADHD, and healthy controls. In each analysis gender, age, scan site, Conners’ score at scan time (T1), and head motion during scanning (calculated as mean frame-wise displacement) were modeled as covariates of no interest.

To investigate whether the change in ADHD symptoms over time was associated with prefrontal functioning, we primarily analyzed the rfMRI data using the dimensional approach. Therefore, measures of change were calculated for the hyperactive/impulsive and inattentive symptoms separately using Conners’ standardized T-score. The score at follow-up was subtracted from the baseline score and subsequently divided by the baseline score [(T1 – T2)/T1]. Through dividing by the baseline score the change in Conners’ score was expressed as a percentage of the baseline score, correcting for possibly confounding variation at baseline. We conducted a linear regression analysis with RSFC of the executive control network as dependent variable and symptom change measure as predictor; this was done for each of the change scores (hyperactive/impulsive and inattentive symptoms) separately. Note that as we included the Conner’s score at T3 as a covariate in all analyses (as indicated above), we controlled for variance related to diagnostic status at T2.

Next, we investigated whether a cerebellar and/or subcortical RSFC deficit was present in both remittent and persistent ADHD compared to healthy control groups. Therefore, we applied a categorical group comparison directly comparing non-cortical RSFC of the three groups. Groups were formed based on the scores from the Conners’ questionnaires at follow-up (see Supplement for more information). In short, participants belonging to the persistent ADHD group received a T-score ≥ 63 on both DSM hyperactive/impulsive and inattentive subscales of Conners’ ADHD questionnaire at both baseline and follow-up. ADHD participants not meeting criteria for persistence were categorized as remitted. Control participants were required to receive T-scores < 63 on the two scales of each Conners’ ADHD questionnaire at both baseline and follow-up.

Finally, we investigated inter-network connectivity through conducting both dimensional and categorical analysis on correlation scores derived by correlating the timeseries obtained for the executive network and each of the subcortical seed regions.

All comparisons were conducted using non-parametric randomization techniques (applying 5000 permutations, FSL-Randomize) with threshold-free cluster enhancement [TFCE; (S. Smith & Nichols, 2009)]. Statistical significance was determined by means of a family-wise error (FWE) threshold of p < .05, corrected for multiple comparisons. To check whether the results were influenced by scan site, gender, age, duration of medication use, or ODD/CD comorbidities, we conducted post-hoc sensitivity analyses. Demographic differences between remittent ADHD, persistent ADHD, and healthy control groups were tested using F-tests for continuous variables and X²-tests for categorical variables.

3. Results

3.1. Clinical outcome

Demographic and descriptive data are presented in Table 1. Both hyperactive/impulsive and inattentive behavior decreased significantly over time [respectively, t(127) = 7.244, p < .001; t(127) = 6.348, p < .001], however, remittance was skewed towards the hyperactive/impulsive domain. As expected, persistent and remittent groups differed in ADHD symptoms at follow-up and in hyperactive/impulsive symptoms at baseline, with the persistent group reporting more symptoms than the remittent group. The difference at baseline was modest [t(127) = -2.4, p = .02, Cohen’s d = .42]. We included baseline score in the change measure to correct for the individual variation at baseline. In line with previous reports (Biederman et al., 2011), participants with persisting symptoms showed higher rates of comorbid ODD compared to participants with remitting symptoms. No significant correlations were found between change in hyperactive/impulsive or inattentive symptoms and age (r = .12) or duration of medication use (r = .11 and r = .06). Neither was duration of medication use correlated to Conners’ score at follow-up (r = .14).

3.2. Resting state networks

3.2.1. Executive control network

First, we examined the relationship between the developmental improvement in ADHD symptoms and RSFC of the executive control network. Confirming our hypothesis, a greater decrease in hyperactive/impulsive ADHD symptoms was associated with higher RSFC within the anterior cingulate and paracingulate gyrus (Fig. 2 and Table 2). This finding suggests a stronger integration of these frontal regions in the executive control network. We observed no significant associations between developmental changes in inattentive symptoms and RSFC of the executive control network (Fig. S1). Post-hoc sensitivity analyses showed (1) similar results when
extreme subjects were excluded; (2) no association between executive control RSFC and age or duration of medication use; (3) similar significant results with IQ or comorbid diagnosis of ODD/CD added to the model; (4) same direction of effects at both scan locations, in both genders, and in participants with ADHD only; (5) no difference in performance on neuropsychological tasks at baseline between participants with remittent and persistent ADHD. Tables and figures illustrating the sensitivity analyses can be found in the Supplementary materials.

Results of a categorical comparison of executive control RSFC between the remittent ADHD, persistent ADHD, and healthy controls are in line with the dimensional analysis. The remittent ADHD group showed higher RSFC in prefrontal regions of the executive control network compared to healthy controls. The results encompassed the same regions as the results of the dimensional analysis and spread out to bilateral regions (a more detailed description of this analysis and its results can be found in the Supplementary materials and Supplementary Fig. 57).

### Inter-network connectivity

We conducted a dimensional analysis to investigate whether the connectivity between the executive control and each subcortical network was related to the ADHD change scores (as calculated above) and a categorical analysis to investigate whether the between network connectivity differed between ADHD (persistent and remittent) and control groups. Neither the dimensional analysis, nor the categorical analysis yielded significant results.

### Discussion

In a longitudinal follow-up study including 129 participants with ADHD and 100 healthy controls, we tested the neurodevelopmental theory of Halperin and Schulz (2006). This theory states that remission of ADHD results from improved prefrontal top-down control, while ADHD, independent of the current status, is characterized by stable non-cortical deficits. We demonstrated that a decrease in hyperactive/impulsive ADHD symptoms was associated with stronger functional
connectivity in frontal regions of the executive control network. In line with these results, a complementary group comparison showed that participants with remitting ADHD symptoms had higher frontal functional connectivity than healthy controls, while persistent ADHD cases exhibited connectivity intermediate to remittent ADHD cases and healthy controls. Both analyses indicated a stronger integration of frontal regions within the executive control network in remittent ADHD, suggesting a compensatory mechanism for part of the ADHD pathology (Johnson, 2012) (see further below). We were unable to confirm the hypothesis that non-cortical dysfunctions are related to ADHD pathology.

Confirming the neurodevelopmental theory, a developmental decrease in hyperactive/impulsive symptoms was associated with stronger integration of the ACC within the executive control network. This finding is in line with several studies that have implicated prefrontal regions, including ACC, in ADHD. Specifically, hypo-activation of ACC during cognitively demanding tasks (Bush, 2010; Bush et al., 1999; Cortese et al., 2012; Hart et al., 2012), and aberrant connectivity of ACC during rest have been reported in participants with ADHD (for review see (Oldehinkel, Franx, Beckmann, Buitelaar, & Mennes, 2013)). Furthermore, the development of functional connectivity of ACC has also been demonstrated

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Table 2 — Peak voxels and localization of significant clusters of categorical and dimensional analysis.

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</tbody>
</table>

Fig. 2 — Significant associations between RSFC of the executive control network and change in hyperactive/impulsive scores. Higher RSFC was associated with a larger decrease in hyperactive/impulsive behavior.
to be deviant in ADHD. While in healthy controls negative connectivity between ACC and precuneus increased with age, such a relationship was absent in participants with ADHD (Sun et al., 2012). Finally, our results are in line with the idea that the PFC of participants with ADHD is less flexible in recruiting other brain areas necessary to perform specific cognitive tasks (Fassbender & Schweitzer, 2005).

When grouping participants into the traditionally used persistent ADHD, remitted ADHD, and healthy control categories, we observed that participants with remitted ADHD exhibited significantly higher RSFC within the executive control network compared to healthy controls. The current pattern of results is more consistent with the presence of mechanisms that compensate for the ADHD deficit (Fassbender & Schweitzer, 2006; Johnson, 2012), than with the idea that participants with remitted ADHD would show connectivity similar to healthy controls (i.e., normalization). We propose that while hyper-connectivity would be inefficient for healthy controls, it might be an efficient mechanism to suppress ADHD symptoms related to higher order executive functioning in participants with ADHD. These results corroborate earlier findings suggesting prefrontal compensation during inhibition (Schulz, Newcorn, Fan, Tang, & Halperin, 2005; Suskauer et al., 2008). Although participants with persistent ADHD also showed a developmental decrease in symptoms, they did not exhibit significantly different RSFC from either the healthy controls or participants with remitted ADHD. The smaller decrease in symptoms in the persistent ADHD group compared to the remitted ADHD group may thus be associated with insufficient prefrontal compensation to cope with ADHD behavior. This interpretation highlights the importance of assessing ADHD from a dimensional perspective, in addition to the classic categorical approach to compare patient groups with healthy controls (Chabernaud et al., 2012; Insel et al., 2010; Lubke, Hudziak, Derks, van Bijsterveldt, & Boomsma, 2009; Forderman et al., 2007). A dimensional diagnostic approach has the advantage that there is no need to choose an arbitrary clinical threshold to define separate groups. However, an oversimplification of ADHD as an extreme on the behavioral axis may not be appropriate, as qualitative differences in dysfunction might also be present, e.g., involvement of different brain regions (Chabernaud et al., 2012).

In contrast to our hypothesis and the Halperin and Schulz theory, we did not find support for non-cortical dysfunctions in ADHD. However, the theory does not formulate specifically which non-cortical dysfunctions or brain areas underlie ADHD. We investigated cerebellar and subcortical RSFC, but it might be possible that non-cortical deficits are to be found in even more basal brain structures such as the brain stem. This area of the brain, however, is difficult to image in high quality using MRI due to cardiogenic noise (Beissner, Deichmann, & Baudrexel, 2011; Greitz et al., 1992). In addition, the absence of a deficit in non-cortical resting state connectivity does not imply the absence of any cerebellar or subcortical deficits, as other neural properties can be affected. For example, volumetric differences in cerebellar and subcortical regions have been related to ADHD pathology (Mackie et al., 2007; Seidman, Valera, & Makris, 2005; Valera et al., 2007). Furthermore, an increasing number of studies does not point to one core brain deficit in ADHD, but instead describes widespread neural abnormalities associated with the disorder (Bush, 2010; Coghill, Hayward, Rhodes, Grimmer, & Matthews, 2014; Durston, 2003; Fair, Bathula, Nikolas, & Nigg, 2012; Sonuga-Barke, 2005). This in turn, may underlie the heterogeneity of neurocognitive deficits in individuals with ADHD (Nigg, Willcutt, Doyle, & Sonuga-Barke, 2005; Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005).

We followed a large and well-phenotyped sample of children over a period of 6 years allowing us to test an influential neurodevelopmental theory (Halperin & Schulz, 2006). However, our findings should be interpreted in within the following boundary conditions. First, although diagnostic data were available at two time points, MRI measurements were available at follow-up only. Therefore, we can only speculate as to the RSFC status of participants at baseline. In any case and of note, we assessed cognitive functioning by neuropsychological tests at baseline and did not find differences between participants with remitting and persisting ADHD. We refer to the Supplementary material accompanying this manuscript for further details on this analysis. Second, developmental changes in inattentive behavior were minimal, which could explain why RSFC was not related to changes in inattentive symptoms. A follow-up assessment at an older age might allow for larger changes in inattentive behavior. Third, the vast majority of participants with ADHD had a stimulant treatment history. As such it possible that long-term medication use might have influenced our results. However, in our sample, there was no difference in medication use between remitted and persistent ADHD cases. In addition, we attempted to exclude acute effects of medication on brain function as all participants withheld stimulant medication from 48 h before the acquisition of the fMRI scan. Further sensitivity analyses showed no association between the duration of medication use and the strength of the executive control RSFC. Finally, this was a hypothesis-driven study in which we specifically tested the neurodevelopmental model proposed by Halperin and Schulz (2006). This limited us to examine the selected networks; future research is warranted to investigate other networks, as ADHD has frequently been related to abnormalities in the default mode network connectivity (Broyd et al., 2005; Castellanos et al., 2008). Furthermore, a previous study investigating remission of ADHD revealed that participants with persistent ADHD showed abnormal connectivity between regions belonging to the default mode network (Mattfeld et al., 2014).

In summary, a greater developmental decrease in ADHD symptoms, and hyperactivity/impulsivity symptoms in particular, was associated with higher connectivity of frontal regions in the executive control network, indicating better integration within this network. This finding is in line with a developmental theory posited by Halperin and Schulz (2006) that proposes a compensatory role for PFC in the symptomatic remission of ADHD. In contrast, we could not confirm the existence of aberrant non-cortical RSFC in ADHD, independent of current diagnosis.

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Declaration of interest

Jan Buitelaar has been in the past three years a consultant to/ member of advisory board of and/or speaker for Janssen Cilag BV, Eli Lilly, Bristol-Myer Squibb, Shering Plough, UCB, Shire, Novartis and Servier. He is not an employee of any of these companies and not a stock shareholder of any of these companies. He has no other financial or material support, including expert testimony, patents or royalties. Jaap Oosterlaan has been on the advisory board of Shire and UCB Pharmaceuticals. He has received an unrestricted grant from Shire. Pieter Hoekstra has received honoraria for advice from Eli Lilly and Shire. The other authors have no potentially competing interests.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cortex.2015.08.012.

References


White matter microstructure and developmental improvement of hyperactive/impulsive symptoms in Attention-Deficit/Hyperactivity Disorder

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Background: A developmental improvement of symptoms in Attention-Deficit/Hyperactivity Disorder (ADHD) is frequently reported, but the underlying neurobiological substrate has not been identified. The aim of this study was to determine whether white matter microstructure is related to developmental improvement of ADHD symptoms.

Methods: A cross-sectional Magnetic Resonance Imaging (MRI) analysis was embedded in a prospective follow-up of an adolescent cohort of ADHD and control subjects (NeuroIMAGE). Mean age at baseline was 11.9 years, mean interval of follow-up was 5.9 years. About 75.3% of the original cohort was retained successfully. Data of 101 participants with ADHD combined type at baseline and 40 healthy controls were analysed. ADHD symptoms were measured with semistructured, investigator-based interviews and Conners’ questionnaires, on the basis of DSM-IV criteria. Fractional anisotropy (FA) and mean diffusivity (MD) indices of white matter microstructure were measured using whole brain diffusion tensor imaging at follow-up only. In a dimensional analysis FA and MD were related to criteria. Fractional anisotropy (FA) and mean diffusivity (MD) indices of white matter microstructure were measured using whole brain diffusion tensor imaging at follow-up only. In a dimensional analysis FA and MD were related to change in ADHD symptoms. To link this analysis to DSM-IV diagnoses, a post hoc categorical group analysis was conducted comparing participants with persistent (n = 59) versus remittent (n = 42) ADHD and controls. Results: Over time, participants with ADHD showed improvement mainly in hyperactive/impulsive symptoms. This improvement was associated with lower FA and higher MD values in the left corticospinal tract at follow-up. Findings of the dimensional and the categorical analysis strongly converged. Changes in inattentive symptoms over time were minimal and not related to white matter microstructure. Conclusions: The corticospinal tract is important in the control of voluntary movements, suggesting the importance of the motor system in the persistence of hyperactive/impulsive symptoms. Keywords: Attention-Deficit/Hyperactivity Disorder, hyperactivity/impulsivity, white matter, diffusion tensor imaging, recovery, development.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a common neurodevelopmental disorder characterized by excessive levels of inattention and/or hyperactivity and impulsivity (American Psychiatric Association, 2000). Childhood onset ADHD persists into adulthood in 15%–65% of cases, depending on the definition of persistence (Barkley, Fischer, Smallish, & Fletcher, 2002; Biederman, Petty, Evans, Small, & Faraone, 2010; Faraone, Biederman, & Mick, 2006; Mannuzza, Klein, & Moulton, 2003). Hyperactive/impulsive symptoms are found to decline at a higher rate than inattentive symptoms (Biederman, Mick, & Faraone, 2000). Although various psychiatric factors have been associated with persistence of ADHD into adulthood (Biederman et al., 1996, 2000; Mick et al., 2011), relatively little is known about associated biological mechanisms. A neurodevelopmental theory associates improvement of ADHD symptoms with the development of the prefrontal cortex and related top-down executive control (Halperin & Schulz, 2006). Within this theory, a noncortical dysfunction is hypothesized to be static over time in all patients with a childhood ADHD diagnosis. Behavioural studies examining the relation between executive control functioning and remission of ADHD reported inconsistent results (Biederman et al., 2000; Fischer, Barkley, Smallish, & Fletcher, 2005; Halperin, Trampush, Miller, Marks, & Newcorn, 2008; Mick et al., 2011).

Few studies have examined the neurobiological underpinnings of remission of ADHD. A functional MRI study reported lower thalamo-cortical activation during response preparation for a cued reaction time task in adults with childhood ADHD (Clerkin et al., 2013). These findings suggested dysfunction of the thalamus in both remitters and persisters. Stronger functional integration of the thalamo-cortical network did parallel symptom recovery, supporting the

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neurodevelopmental theory. A structural brain imaging study reported that remission of symptoms was associated with normalization of the developmental trajectory of cortical thickness, particularly in the right parietal cortex (Shaw et al., 2006). Persistent ADHD was characterized by a fixed thinning of the medial prefrontal cortex (Prola et al., 2011; Shaw et al., 2006), indicating a role of the prefrontal cortex in the improvement of ADHD symptoms.

In recent years, neural models of ADHD are shifting focus from the study of regional brain abnormalities to disturbed connectivity in networks (Konrad & Eickhoff, 2010). Structural connectivity and white matter (WM) abnormalities are key elements in these models. An established modality for investigating WM microstructure is diffusion tensor imaging (DTI; Basser, Mattiello, & LeBihan, 1994). Two commonly used diffusion tensor derivative measures are mean diffusivity (MD), which measures the magnitude of diffusion and fractional anisotropy (FA), which quantifies the directionality of diffusion. Cross-sectional DTI studies in children and adults with ADHD have shown inconsistent results. A recent meta-analysis of DTI studies in ADHD which dealt mainly with children, reported WM abnormalities in the anterior corona radiata, cerebellum, internal capsule and forceps minor (van Ewijk, Heslenfeld, Zwiers, Buitelaar, & Oosterlaan, 2012). DTI studies of adults with persistent ADHD revealed abnormal WM microstructure in the temporal, orbitomedial prefrontal lobe and right anterior cingulate bundle (Casey et al., 2007; Konrad et al., 2010; Makris et al., 2008). The only study to date that has investigated the role of WM structure in remission of ADHD reported lower FA values in several WM tracts in ADHD subjects compared to controls, but no differences in DTI measures between persisters and remitters (Cortese et al., 2013).

In this study, we aimed to investigate whether developmental improvement of ADHD symptoms is linked to the microstructure of WM tracts. To that end, a large childhood ADHD cohort was followed longitudinally over 6 years. The association between developmental improvement of ADHD symptoms over adolescence and WM microstructure at follow-up was examined in a dimensional analysis. This analysis was corroborated with a categorical analysis that compared remitters and persisters head-to-head while also using a healthy control (HC) group. In accordance with the neurodevelopmental model, we hypothesized that developmental improvement of ADHD symptoms would be related to the WM microstructure of the fronto-striatal circuitry involved in executive control. Furthermore, we expected WM microstructure (a) of participants with remitting ADHD to be similar to HCs, and (b) of participants with persistent ADHD to differ from WM of both remitting ADHD and HCs.

Methods

Participants

Subjects with ADHD combined type (ADHD-C) and HCs participated in the Dutch part of the International Multicenter ADHD Genetics study (Muller et al., 2011a,b). In short, all children were Caucasian, between 6 and 18 years (M = 11.9, SD = 2.6) and with an IQ $\geq$ 70. Exclusion criteria were a diagnosis of autism, epilepsy, brain, or genetic disorders. IQ was estimated based on the WISC or WAIS–III vocabulary and block design subtests at baseline and at follow-up. Extensive diagnostic, neurocognitive and genetic data were collected at the VU University Amsterdam and Radboud University Medical Centre in Nijmegen. All participants were invited for follow-up measurement with a mean follow-up period of 5.9 years (SD = 0.6). At the second assessment, a similar phenotypic protocol was followed and complemented with the acquisition of MRI brain scans.

In the current analyses we included all subjects diagnosed with ADHD-C at baseline and a DTI-scan of high quality at follow-up ($n = 101$; Figure S1). There were no differences between the subjects included in current analysis and the complete sample on measures of ADHD severity ($p = .935$), age ($p = .206$) and gender ($p = .134$). Forty age-matched HC subjects were additionally included, they were recruited from schools and did not meet criteria for ADHD, neither did their first-degree relatives. The study was approved by the regional ethics committee and written informed consent was given by the children and their parents.

Diagnostic assessment

Diagnosis was based on a semi-structured, investigator-based interview and Conners’ questionnaires. The interview was the Dutch version of the Parental Account of Children’s Symptoms (PACS; Taylor, 1991; Taylor, Schachar, Thorley, & Wieselberg, 1986) at baseline and the Schedule for Affective Disorders and Schizophrenia for Children (K-SADS; Kaufman et al., 1997) at follow-up. Both interviews are compatible with the DSM-IV-TR (American Psychiatric Association, 2000). Initially, all participants were administered the screening interview. Participants with elevated scores on any of the screen items were administered the full ADHD supplement. The Conners’ questionnaires were the teacher report [Conners’ Teacher Rating Scale: Long version (CTRS-R:L)] applied for children $\leq 18$ years or a self-report [Conners’ Adult ADHD Rating Scales-Self-Report: Long version (CAARS-S:L)] applied for children $>18$ years (Conners et al., 1997; Conners, Sitarenios, Parker, & Epstein, 1998). Using a diagnostic algorithm, a combined symptom count was calculated by adding symptom counts on the interview and CTRS-R:L (for participants $<18$) or CAARS-S-L (for participants $\geq 18$), both providing operational definitions of each of the 18 behavioural symptoms defined by the DSM-IV (American Psychiatric Association, 2000). Symptoms of the Conners’ questionnaires were only added to the combined symptom count if at least two symptoms were reported, to avoid the Conners’ score to put too much weight on the diagnosis. Of the Conners’ ADHD questionnaires, the following scales were used: DSM Inattentive behaviour (Scale L of the CPRS-R-L/CTRS-R-L; Scale E of the CAARS-S-L), DSM Hyperactive/Impulsive behaviour (Scale M of the CPRS-R-L/CTRS-R-L; Scale F of the CAARS-S-L) and DSM Total (Scale N of the CPRS-R-L/CTRS-R-L; Scale G of the CAARS-S-L). For participants using medication, ratings were done of children’s functioning off medication.

For the dimensional analyses, we calculated a measure of symptom change over time by subtracting the symptom count at baseline from the count at follow-up. For the categorical analyses, participants with a combined symptom count of $\geq 6$ symptoms of hyperactive/impulsive and/or inattentive behaviour were diagnosed with persistent ADHD, provided they (a)
met the DSM-IV criteria for pervasiveness and impact of the disorder, (b) had an age of onset before 7 and (c) received a standardized T-score ≥63 on at least one DSM ADHD scale of the ADHD questionnaire. ADHD participants not meeting criteria for persistence were categorized as remitted. Completely remitted participants and controls had to score T < 63 on all scales of the ADHD questionnaire and had ≤3 symptoms derived from the combined symptom counts of the K-SADS and CTRS-R:L/CAARS-S:L.

**Diffusion tensor imaging and (pre-) processing**

MRI data were acquired at follow-up only with 1.5 T scanners from Siemens (MAGNETOM-Sonata and AVANTO) at two scan locations. Both scanners were equipped with the same 8-channel phased-array head coil. For each participant, whole brain diffusion-weighted images were collected (twice refocused PGSE EPI; 60 diffusion-weighted directions; b-factor 1,000s/mm²; 5 nondiffusion-weighted images; interleaved slice acquisition; TE/TR = 97/8,500 ms; GRAPPA-acceleration 2; phase full Fourier; voxel size 2.0 × 2.0 × 2.2 mm). DTI images were realigned and corrected for residual eddy-current (SPM8; http://www.fil.ion.ucl.ac.uk/spm, London, UK) and for artefacts from head and/or cardiac motion using robust tensor modelling (PATCH; Zwiets, 2010). All DTI-scans were visually inspected to assess quality of the data. When the quality was insufficient, the data of the subject were excluded (n = 21, see Figure S1). Diffusion tensors and derived FA and MD values were then calculated for each voxel (FSL 4.1.7; Behrens et al., 2003).

The group analysis of diffusion parameters was performed using Tract-Based Spatial Statistics (FSL TBSS; Smith et al., 2006). FA and MD images of each participant were nonlinearly registered to the FMRIB-S8_FA template (MN152-space). Subsequently, a group mean FA-image was created to produce a mean skeleton map of WM tracts. Finally, diffusion parameters of each subject were projected onto the group skeleton, which had at least 50 samples passing through them (out of 5,000 generated streamlines). Finally, the individual thresholded images were projected into standard space, binarized, summed over individuals and thresholded to include tracts that were present in at least 75% of the subjects (Boorman, O'Shea, Sebastian, Rushworth, & Johansen-Berg, 2007).

**Probabilistic tractography**

To visualize the most likely pathways extending from significant FA clusters, we estimated voxel-wise fibre orientation distributions and probabilistic streamline tractography (FSL-Probtrackx; Behrens et al., 2003). Significant voxels of the TBSS analysis were mapped into each subject’s native space and used as seed-masks for tractography. To remove spurious connections, the resulting connectivity maps of individual subjects were thresholded to include only voxels which had at least 50 samples passing through them (out of 5,000 generated streamlines). Finally, the individual thresholded images were projected into standard space, binarized, summed over individuals and thresholded to include tracts that were present in at least 75% of the subjects (Boorman, O’Shea, Sebastian, Rushworth, & Johansen-Berg, 2007).

**Statistical analysis**

Demographic between-group differences were tested using F-tests for continuous variables and χ²-tests for categorical variables. For both the dimensional as the categorical MRI analysis, general linear models (GLMs) were built with FA or MD values as dependent variable and gender, age, duration of medication use and scanner site as covariates of no interest. We corrected for multiple comparisons by implementing threshold-free cluster enhancement (FSL-TFCE; Smith & Nichols, 2009).

**Dimensional analysis: symptom change over time.** In this within-group analysis among subjects with ADHD (n = 101; Table 1) we tested voxel-wise whether WM microstructure differed as a function of hyperactive/impulsive and inattention symptom change. Therefore, univariate linear regressions were run with FA or MD values as dependent variable, symptom change as predictor variable and symptom count at follow-up as additional nuisance regressor to correct for differences in WM related to symptom count at scan time.

**Categorical ROI analysis: persistent and remittent groups in relation to HCs.** To link our analysis to traditional DSM-IV diagnoses and relate our findings to previous reports, we divided the same subjects as in the dimensional analysis into persisters (n = 59) and remitters (n = 42) based on the hyperactive/impulsive symptom count at follow-up and added an age-matched HC group (n = 40; Table 2). Significant clusters of the dimensional analysis were used as regions of interest (ROIs) to conduct a voxel-wise comparison between persisters, remitters and HC. FA and MD of the three groups was compared using an ANCOVA with gender, age, duration of medication use and scanner site as covariates of no interest.

**Results**

**Clinical outcome**

Tables 1 and 2 present the demographics of the sample. Both the hyperactive/impulsive and the inattentive symptom count were significantly lower at follow-up compared to baseline. Remittance was strongly skewed towards the hyperactive/impulsive domain of ADHD, with a mean symptom change of −2.30 symptoms (SD = 2.37), and less towards the inattentive symptoms (mean change: −1.02 symptoms, SD = 1.65). In fact, only n = 3 (3%) of the childhood ADHD sample were completely remitted.

**Table 1** Demographic and clinical characteristics for the Attention-Deficit/Hyperactivity Disorder group at T1 and T2

<table>
<thead>
<tr>
<th></th>
<th>T1</th>
<th>T2</th>
<th>Test statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>101</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>11.9 (2.7)</td>
<td>17.8 (2.7)</td>
<td>t(100) = 77.494**</td>
</tr>
<tr>
<td>Estimated IQ</td>
<td>95.4 (13.4)</td>
<td>95.0 (14.2)</td>
<td>t(76) = −0.605</td>
</tr>
<tr>
<td>Clinical Interview</td>
<td>8.1 (1.0)</td>
<td>5.8 (2.4)</td>
<td>t(100) = 9.763**</td>
</tr>
<tr>
<td>hyperactive*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interview inattentive*</td>
<td>8.2 (1.0)</td>
<td>7.1 (1.6)</td>
<td>t(100) = 6.251**</td>
</tr>
</tbody>
</table>

M (SD); Estimated IQ based on Wechsler Intelligence Scale for Children or Wechsler Adult Intelligence Scale—III Vocabulary and block design; estimated IQ missing for 24 subjects at baseline.

*Symptom count. Maximal nine symptoms per dimension (≥6 is clinical threshold).

*p < .05; **p < .001.
We therefore focused on changes in the hyperactive/impulsive symptom count in the analyses. Persistent and remitted ADHD groups did not differ significantly in hyperactive/impulsive symptom counts at baseline. As expected, there were differences between the groups in terms of hyperactive/impulsive symptom score at follow-up and change in symptoms over time (Table 2). Between baseline and follow-up 86.1% of the subjects with ADHD used methylphenidate and 2.0% dexamphetamine. Pearson correlations between the change in symptoms and age at follow-up, r(99) = -.07, p = .466, duration of medication use, r(99) = .03, p = .751, or baseline symptom count, r(99) = .2, p = .796, were low in the ADHD cases. No differences in follow-up interval were found between the persistent and remitted ADHD groups.

**MRI results**

**Dimensional analysis: symptom change over time.** FA values varied as a function of hyperactive/impulsive symptom change over age in the left superior longitudinal fasciculus and corona radiata (peak voxel: t = 4.84, p = .03; Figure 1 upper panel, Table 3). Lower FA values were associated with a stronger decrease (i.e. remission) in hyperactive/impulsive symptoms (Figure 2, left). Probabilistic tractography using significant FA clusters as seed regions indicated that these regions were most likely part of the corticospinal tract, running from the motor cortex through the corona radiata into the internal capsule (Figure 1, middle panel). MD values also varied as a function of hyperactive/impulsive symptom change (Figure 1, lower panel). Higher MD values were associated with stronger remission of hyperactive/impulsive symptoms (Figure 2, right), and significant clusters were located in similar regions as the FA results. We identified these regions as the bilateral corona radiata and corona radiata (peak voxel: t = 4.78, p = .04) and superior longitudinal fasciculus (peak voxel: t = 4.77, p = .02), extending to the internal and external capsula, corpus callosum (genu, body and splenium) and cingulum.

Change in inattentive symptom count was not found to be significantly associated with altered FA
or MD values. A series of control and sensitivity analyses showed that: (a) Neither FA nor MD values were found to be associated with IQ or medication use; (b) Adding comorbid ODD/CD diagnosis to or removing symptom counts at follow-up from the model did not significantly change the results; (c) Results were replicated (although not reaching significance due to reduced statistical power) in each scan site separately and in two age-groups based on a median split (at the age of 17.9); and (d) No interaction effects were found between the change in symptoms and medication use, gender, or age in predicting FA and MD values (see also Figure S2).

**Categorical ROI analysis: persistent and remittent groups in relation to HCs**

In the ROIs, created based on the results of the dimensional analyses, both the HC and remittent group exhibited lower FA and higher MD values compared to the persistent hyperactive/impulsive group (Table 3; Figure 3). The remittent
Discussion
We investigated the relationship between WM microstructure and developmental improvement of ADHD symptoms in a longitudinal study of a large childhood ADHD cohort. Consistent with previous reports we observed that hyperactive/impulsive symptoms improved to a greater extent than inattentive symptoms (Biederman et al., 2000; Hart, Lahey, Loeber, Applegate, & Frick, 1995). Complete remission was rare. Hence, we focused our analyses on the WM correlates of improvement in hyperactive/impulsive symptoms. Persistence of diagnostic status was associated with higher FA and lower MD values in the left superior longitudinal fasciculus and corona radiata, which are part of the corticospinal tract. These motor-associated changes might reflect the findings of a stronger remittance of hyperactive symptoms compared to enduring attention deficits (Biederman et al., 2000; Lahey et al., 1994, 2004; Molina et al., 2009). Also, in these regions, FA and MD values of subjects with remittent hyperactive/impulsive symptoms did not differ from those of HC, while subjects with persistent symptoms did show higher FA and lower MD values in this region compared to both remitters and HC. This is consistent with and an extension of our previous findings showing that higher FA and lower MD are widely associated with more behavioural ADHD symptoms (van Ewijk et al., 2014).

In contrast to our hypothesis, no associations were found between the developmental improvement of ADHD symptoms and microstructure of fronto-striatal WM tracts. Importantly, this does not imply the absence of associations between fronto-striatal grey matter and ADHD remission. Although not hypothesised, the change in hyperactive/impulsive symptoms was related to WM microstructure of the corticospinal tract. This tract is of interest because of the motor hyperactivity subjects with ADHD exhibit and the essential role this tract plays for the motor system. It contains fibres running from the primary motor, premotor, supplementary motor, somatosensory, parietal and cingulate cortex to the spine and, thus, is involved in the control of posture,

![Figure 2](image1.png)

**Figure 2** Graphs illustrating the relationship between (Left) mean fractional anisotropy (FA) values extracted from the significant results of the dimensional analysis and change in hyperactive/impulsive symptom count; (Right) mean diffusion (MD) values and change in hyperactive/impulsive symptom count

![Figure 3](image2.png)

**Figure 3** Tukey box plots illustrating the results of the categorical group comparison between persistent, remittent and healthy control groups. (Left) mean fractional anisotropy (FA) values for the three groups; (Right) mean diffusion (MD) values for the three groups (*p < 0.05)
but also the control of more complex voluntary movements (Rizzolatti & Luppino, 2001). Our findings were unilateral, which may stem from the fact that 89% of our subjects were right-handed (note that motor axons cross to the contralateral side before reaching the lower motor neurons). Our data do not allow us to make inferences about causality; however, we could speculate that the findings in the corticospinal tract might be down-stream results of improved prefrontal executive control in remitting subjects with ADHD, which in turn may result in less stimulation of these motor tracts.

Although it is generally difficult to interpret FA or MD in terms of the individual elements that constitute the tissue microstructure (Beaulieu, 2002), our higher FA in conjunction with lower MD findings associated with persistent hyperactive/impulsive symptoms may indicate higher efficiency in motor signal transmission, either because the associated axonal fibres are more densely myelinated or because they are more numerous. However, the higher FA values can also be attributed to decreased neuronal branching (Suzuki, Matsuzawa, Kwee, & Nakada, 2003) in brain areas where healthy subjects have many crossing fibres (Mori, Wakana, & Van Zijl, 2005), though this is less likely to be accompanied by lower MD values. The finding of higher FA values in subjects with ADHD compared to controls has been frequently reported for various regions, including the superior longitudinal fasciculus, anterior corona radiata, uncinate fasciculus and thalamic radiation (Davenport, Karatekin, White, & Lim, 2010; Konrad et al., 2010; Silk, Vance, Rinehart, Bradshaw, & Cunnington, 2009; Tamm, Barnea-Goraly, & Reiss, 2012). However, there is a large degree of heterogeneity in DTI results, with studies also reporting lower FA in various regions including the internal capsule, corona radiata and corpus callosum in ADHD compared to control subjects (van Ewijk et al., 2012). The current results differ from those of a recent study using a longitudinal design in adults where the investigators found no difference in WM properties between remitters and persisters (Cortese et al., 2013). This may be explained by the relatively small statistical power given the sample size of their groups (persisters: $n = 15$, remitters: $n = 25$) and the variation in the ADHD subtypes of the persisters (six inattentive, six hyperactive/impulsive and three combined).

Although this study was based on a prospective longitudinal design, brain structure was assessed at follow-up only, hence we can just speculate as to the WM status at baseline. It is possible that differences between subjects with remittent and persistent hyperactive/impulsive symptoms were already present at baseline; in this case, the corticospinal WM may be an early marker of remission of hyperactive/impulsive symptoms over time. Another possibility is that no WM differences between subjects with remittent and persistent symptoms were present at baseline. This implies normalization of the corticospinal WM of subjects with remittent hyperactive/impulsive symptoms.

In this study we followed a large sample of children with ADHD-C over a period of 6 years. This period covers a developmental window that is ideally suited to the investigation of neural correlates of hyperactive/impulsive symptom change over time. However, there are a number of limitations to the study. Diagnostic interviews differed between baseline and follow-up, however, Conner’s questionnaires were used both at baseline and follow-up, and for consistency symptoms were always counted according to DSM-IV criteria. Secondly, this was an observational study in which the vast majority of ADHD participants were taking stimulant medications. More in depth medication studies are warranted to determine the precise effects of medication use on WM microstructure in relation to persistence and remission. Lastly, follow-up assessments at later age may allow for larger developmental change of inattentive symptoms and investigation of the neural underpinnings of this change.

In conclusion, we found in the current ADHD cohort foremost developmental improvement of hyperactive/impulsive symptoms of ADHD, confirming previous studies. The developmental improvement in hyperactive/impulsive symptoms was related to the WM structure in the left corticospinal tract, which has an important role in the motor system. Participants with remittent symptoms did not show differences in this tract compared to HCs, while both those groups differed significantly from participants with persistent symptoms.

Supporting information
Additional Supporting Information may be found in the online version of this article:

Figure S1. Flow chart of subject withdrawal throughout the longitudinal study.

Figure S2. Graphs illustrating the relationship between mean FA values extracted from the significant results and nuisance variables.

Acknowledgements
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Development of ADHD symptoms and white matter microstructure

Abstract

Background. The developmental trajectory of Attention-deficit/hyperactivity disorder (ADHD) is highly variable. While some individuals show remission of ADHD into adulthood, others do not. Up till now, little is known about the neurobiological mechanisms involved in remission of ADHD. Here, we investigated whether developmental changes in white matter microstructure reflect ADHD outcome.

Methods. A prospective longitudinal MRI study of 36 adolescents with ADHD and 33 without ADHD (mean age at baseline across all participants, 16.8 years). From each participant extensive diagnostic information and two MRI scans (baseline and follow-up, mean interval, 3.4 years) were collected. The developmental changes in white matter indices (FA and MD) across the entire white matter skeleton were compared between ADHD and non-ADHD groups. In addition, persistent (n=16) and remittent ADHD groups (n=20) formed based on the Conners score were compared directly. Furthermore, using a dimensional approach, we investigated whether changes in white matter microstructure were related to changes in ADHD symptoms.

Results. Overall, ADHD symptoms decreased over time. As expected, the remittent ADHD group showed fewer symptoms at follow-up than the persistent ADHD group. However, the remittent ADHD group had still more ADHD symptoms than the non-ADHD group. Change in white matter microstructure did not significantly differ between participants with and without ADHD. Furthermore, change in ADHD score was not significantly related to the change in white matter microstructure as measured using FA and MD indices.

Conclusions. Although the amount of ADHD symptoms declined over time, no association with white matter development was found. White matter microstructure might not be the appropriate measure to assess neurobiological changes related to developmental changes in ADHD symptoms.
Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a common neurodevelopmental disorder identified by age inappropriate levels of attention and/or hyperactivity and impulsivity. Although diagnosed during childhood, ADHD persists into adulthood in 20-40% of the cases (Faraone, Biederman, & Mick, 2006). Symptoms of hyperactivity and impulsivity decrease over time at a younger age and at a higher speed than inattentive symptoms (Biederman, Mick, & Faraone, 2000). Even though ADHD is defined as a neurodevelopmental disorder, the nature and developmental trajectory of the underlying neural deficits remain unclear. Abnormal brain development might already start very early in the prenatal or postnatal period, when cell proliferation and migration occur, continues during childhood together with synaptogenesis and synaptic pruning, and extends through adolescence into young adulthood when further myelination takes place (Nelson & Bloom, 1997). Brain development does not occur at a linear pace or uniformly across the brain. In the healthy population, grey matter develops following a non-linear pattern that is characterized by an increase in brain volume and cortical thickness during childhood and a decrease in cortical thickness during adolescence (Gogtay et al., 2004; Reiss, Abrams, Singer, Ross, & Denckla, 1996). In contrast, the developmental trajectory of white matter is not yet fully known. White matter development continues over a prolonged period of time, with evidence for both linear and non-linear trajectories (Casey, Tottenham, Liston, & Durston, 2005; Giedd et al., 1999; Krogsrud et al., 2015; Lebel & Beaulieu, 2011; Paus et al., 1999). Recently, efforts are made to relate brain developmental trajectories to developmental changes in severity and phenotypic expression of psychiatric disorders. Functional MRI studies comparing remittent and persistent ADHD revealed reduced positive connectivity in the default mode network in persistent ADHD (Mattfeld et al., 2014). Furthermore, both remittent and persistent ADHD showed abnormal connectivity between dorsolateral (dlPFC) and medial PFC (mPFC). The authors speculated that as both groups also showed impaired executive functioning performance, this impairment might be directly related to the abnormal dlPFC-mPFC connectivity (Mattfeld et al., 2014). Another study investigating response preparation revealed in remittent ADHD greater cue-related connectivity fronto-thalamic regions than in persistent ADHD (Clerkin et al., 2013). Furthermore, a structural MRI study with longitudinal imaging data revealed an association between thinning of the medial and dorsolateral prefrontal cortex (PFC) and inattentive symptoms. Participants in remission showed a slower rate of medial/cingulated cortical thinning converging to healthy control levels with aging (Shaw et al., 2013). Next to cortical volume, also cerebellar volume has been associated with
clinical outcome, with a worse clinical outcome relating to decreased cerebellar volume (Mackie et al., 2007).

Cross-sectional studies investigating white matter microstructure and development in ADHD report less inattention to be associated with lower FA in left uncinate and inferior fronto-occipital fasciculus (Shaw et al., 2015). Participants with remittent ADHD did not differ from healthy controls in these regions. However, another study showed that childhood ADHD is at adult age associated with lower FA in tracts involved in higher-level cognitive functions as well as sensory and motor functions, i.e., right superior and posterior corona radiata, superior longitudinal fasciculus and left posterior thalamic radiation, retrolenticular part of internal capsula and sagittal stratum (Cortese et al., 2013). Both remittent and persistent ADHD groups showed lower FA than healthy controls, indicating stable deficits independent of ADHD remission. Finally, in a previous cross-sectional study, we showed that a decrease in hyperactive/impulsive symptoms was associated with higher FA and lower MD in the corticospinal tract (Francx et al., 2015). Although informative, cross-sectional developmental studies allow only limited conclusions to be drawn as subjects are not individually tracked over time.

The relation between ADHD outcome and white matter brain connectivity has only been investigated using cross-sectional studies. Here, we applied a longitudinal design following a group of 36 adolescents with ADHD and 33 without ADHD over approximately 3 years. At two timepoints, all participants underwent a diffusion tensor imaging (DTI) scan and diagnostic evaluation. We applied two approaches to investigate the relation between development in white matter microstructure and ADHD. First, we applied an exploratory whole brain approach investigating whether the developmental changes in white matter microstructure were related to the changes in ADHD severity over time. Further, we specifically investigated changes in tracts connecting regions important for higher cognitive control, e.g., fronto striatal tracts, tracts that frequently have been reported as abnormal in ADHD (Cubillo, Halari, Smith, Taylor, & Rubia, 2012; Konrad & Eickhoff, 2010).

Methods and Materials

Participants
Participants were a subgroup of a large prospective longitudinal study, the International Multicenter ADHD Genetics (IMAGE) cohort, which was established from 2004-2006 (as described previously in Muller et al., 2011a; Muller et al., 2011b; Nijmeijer et al., 2009; Rommelse et al., 2008).
At first enrolment (baseline or t0), participants with ADHD combined type were recruited from outpatient psychiatric or pediatric clinics in The Netherlands. Participants from control families were recruited from schools and did not meet criteria for ADHD, neither did their first-degree relatives. Further inclusion criteria for both groups were an IQ≥70, European Caucasian descent, and no diagnosis of autism, epilepsy, general learning difficulties, brain disorders, or known genetic disorders (such as Fragile X or Down syndrome). Extensive diagnostic, neurocognitive, and genetic data was collected at the VU Amsterdam or at the Radboudumc in Nijmegen. All participants were invited to participate in two follow-up studies during which the same protocol was followed and enriched with MRI data. The first follow-up study (t1 or NeuroIMAGE) occurred between 2009-2011 (as described in (von Rhein et al., 2014)), three years later (mean follow up=3.39 years, SD=0.51) participants from Nijmegen were re-invited (t2 or NeuroIMAGE 2). All participants gave informed consent and the study was approved by local ethical committees.

For current analyses, we selected participants that had complete longitudinal diagnostic data and DTI scans at both t1 and t2. In total this were 84 participants. We excluded participants with DTI scans of bad quality (e.g., signal drop out, image blur) after visual inspection (n=9, of which 1 control) and participants with high head-motion (n=6, of which 2 controls). In our final analyses we thus included 36 participants with ADHD combined type at baseline and 33 participants without ADHD at baseline. The latter included healthy controls and unaffected siblings of participants with ADHD (i.e. who never showed clinical ADHD symptoms themselves).

Diagnostic assessment

To determine ADHD diagnoses at each measurement, all participants in the study were similarly assessed using a combination of Conners' ADHD questionnaires and a semi-structured diagnostic interview. The diagnostic interview changed from the Parental Account of Children’s Symptoms (PACS (Taylor, Schachar, Thorley, & Wieselberg, 1986)) at t0 to the Dutch translation of the Schedule for Affective Disorders and Schizophrenia for School-Age Children (K-SADS (Kaufman et al., 1997)) at t1 and t2. As all participants were at each measurement similarly assessed by a parent-rated Conners’ questionnaire (Conners' Parent Rating Scale - Revised: Long version (CPRS-R:L); Conners, Sitarenios, Parker, & Epstein, 1998), we used this questionnaire data for current longitudinal analyses. Two Conners’ scales were used: DSM-Inattentive behavior (scale L of the CPRS-R:L) and DSM-Hyperactive/Impulsive behavior (scale M of the CPRS-R:L) scales. Scores on the Conners’ questionnaires were highly correlated with symptoms counted based on the K-SADS at t1
(for inattention r=0.77; for hyperactivity/ impulsivity r=0.76). For participants using medication, the participants’ functioning was rated when off medication.

Comorbidity with oppositional defiant disorder (ODD) and conduct disorder (CD) were assessed using the K-SADS at t1. Initially only the screening interview was administered, thereafter participants with elevated scores on any of the screen items were also administered the full section.

**Diffusion tensor imaging and (pre)-processing**

MRI data were acquired at the Donders Centre for Cognitive Neuroimaging in Nijmegen, The Netherlands with a 1.5T AVANTO scanner (Siemens, Erlangen, Germany) equipped with an 8-channel phased-array head coil. At t1 and t2 exact the same procedure was followed. For each participant, whole brain diffusion-weighted images were collected (twice refocused PGSE EPI; 60 diffusion-weighted directions; b-factor 1000s/mm2; 5 non-diffusion-weighted images; interleaved slice acquisition; TE/TR=97/8500ms; GRAPPA-acceleration 2; phase full Fourier; voxelsize 2.0x2.0x2.2mm). All DTI-scans were visually inspected to assess quality of the data. When the quality was insufficient the data of the subject was excluded. DTI images were realigned and corrected for residual eddy-current (SPM8; http://www.fil.ion.ucl.ac.uk/spm, London, UK) using affine transformations and mutual information as a cost function. Artifacts from head and/or cardiac motion were corrected using robust tensor modeling (PATCH (Zwiers, 2010)). Diffusion tensors and derived FA and MD values were calculated for each voxel.

**White matter skeleton analysis**

The group analysis of diffusion parameters was performed using Tract-Based Spatial Statistics (FSL-TBSS (Smith et al., 2006)). FA images of each participant were non-linearly registered to the FMRIB-58_FA template (MNI152-space). Subsequently, a group mean FA-image across all subjects and all time points was created to produce a mean skeleton map of WM-tracts. Finally, FA and MD parameters of each subject were projected onto the group skeleton, which was thresholded at FA≥0.2 to exclude peripheral tracts (Smith et al., 2006). White matter maps of the two time points were subtracted to investigate the change in white matter microstructure over time. Non-parametric permutation tests (5000 random permutations; FSL-randomise) were conducted voxel-wise on the whole brain WM-skeleton, while resulting p-values were corrected for multiple comparisons (p<0.05).

**Fronto-striatal ROI analysis**

Next to an exploratory whole brain analysis, we specifically examined cortico-striatal tracts. The selected ROIs were derived from our previous study on the effect of stimulant
treatment on fronto-striatal connectivity (Schweren et al., under review). In this study, we delineated three fronto-striatal white matter tracts and extracted mean FA and MD values for each hemisphere separate. The dIPFC-striatal, mPFC-striatal, and orbitofrontal-striatal tracts were reconstructed using probabilistic tractography (Probtrackx2). For further explanation we refer to the Supplementary material.

**Statistical analysis**

Demographic between-group differences were tested using $F$-tests for continuous variables and $X^2$-tests for categorical variables. For the MRI analyses, two general linear models (GLMs) were built i.e., one for a categorical analysis and one for a dimensional analysis. FA or MD values were taken as dependent variable, change in hyperactivity/impulsivity or inattentiveness as independent variable and gender and age as covariates of no interest. All comparisons were conducted using non-parametric randomization techniques (applying 5000 permutations, FSL-Randomize) with threshold-free cluster enhancement (FSL-TFCE (Smith & Nichols, 2009)). Statistical significance was determined by means of a family-wise error (FWE) threshold of $p<.05$, corrected for multiple comparisons.

**Categorical analysis: Is change in white matter microstructure different in ADHD and non-ADHD groups?**

To investigate whether ADHD and non-ADHD participants show a different developmental pattern of white matter microstructure, we compared the voxelwise change over time in FA and MD between ADHD and non-ADHD groups. Furthermore, we divided the participants with ADHD into persisters ($n=16$) and remitters ($n=20$) based on the ADHD Conners score ($T>63$: compatible with the DSM-IV-TR (American Psychiatric Association., 2000)) at t2. FA and MD changes over time were compared between the three groups using an ANCOVA with gender and age as covariates of no interest.

**Dimensional analysis: Is change in ADHD score related to changes in white matter microstructure?**

To investigate whether change in ADHD score co-occurs with change in white matter microstructure, we tested whether voxelwise changes in FA and MD were related to changes in hyperactive/impulsive and/or inattention symptoms. Therefore, univariate linear regressions were run with FA or MD values as dependent variable, symptom change as predictor variable and gender and age as covariates of no interest. We tested this within the ADHD group only and in addition, across all participants.
Results

Clinical outcome

Demographic and descriptive data are presented in Table 1. A repeated measures ANOVA determined that mean ADHD score differed significantly between time points, Wilks’ Lambda=0.028, F(4,246)=306.877, p<.001. Post-hoc paired samples t-tests indicated significant differences between t0 (M=63.41, SE=2.05) and t2 (M=56.44, SE=1.17) and between t1 (M=60.537, SE=1.85) and t2. The interaction between time and group was significant [Wilks’ Lambda=0.799, F(4, 250)=3.646, p<.001]. At none of the time points unaffected siblings differed from healthy controls. Persistent and remittent ADHD groups did not differ at baseline, but evolved to be different by t2 (Table 1).

<table>
<thead>
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<tr>
<td>X²(2)= 8.414</td>
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<tr>
<td>F(2,66)=3.297</td>
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<tr>
<td>F(2,66)=31.554**</td>
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<td>F(2,66)=29.838**</td>
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<td>F(2,66)=27.082**</td>
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<td>F(2,66)=27.793**</td>
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<td>F(2,66)=27.933**</td>
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<td>F(2,66)=2.196</td>
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<td>F(2,66)=89.469**</td>
<td>P&gt;R&gt;N</td>
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<td>F(2,66)=88.577**</td>
<td>P&gt;R&gt;N</td>
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Table 1. Demographic and clinical characteristics of the ADHD remittent, ADHD persistent, non-ADHD groups.

<table>
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<td>F(2,66)=89.469**</td>
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<td>F(2,66)=88.577**</td>
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Longitudinal DTI data

The effect of time

A voxelwise comparison of the two time points across all participants did not reveal any statistically significant differences in FA values (p>0.05). The mean FA change in the skeleton was relatively small (M=0.003, SD=0.014). MD changed significantly over time in widespread areas of the skeleton (Figure 2), but the mean change over the skeleton, although significant, was small (M=0.00001, SD=0.00001) (Figure 3).
The interaction effect of group and time

Categorical analysis

Next, we examined the differences between ADHD and non-ADHD groups in white matter microstructure development (Figure 3, 4, and 5). Neither the developmental change in FA, nor MD differed significantly between ADHD and non-ADHD groups. In addition, when splitting the ADHD group up into remittent and persistent ADHD, no significant differences were found in white matter development between the three groups.

Based on previous literature, we specifically investigated cortico-striatal tracts (Cubillo et al., 2012; Konrad & Eickhoff, 2010). Therefore, we extracted mean FA and MD values from the reconstructed tracts, i.e., dorsolateral prefrontal-striatal, medial prefrontal-striatal, and orbitofrontal-striatal tracts. Developmental changes of FA or MD within these tracts were not significantly different between ADHD and non-ADHD groups. No significant interactions between age and group or gender and group were found, and no significant main effect of age (correcting for group) was found.
**Dimensional analysis**

Finally, we investigated the association between developmental changes in white matter microstructure and the change in ADHD scores. The changes in ADHD scores over time were not significantly associated with the change in FA or MD across all participants. Furthermore, no associations were found when we tested this within the ADHD group only.

Figure 4. Change in white matter microstructure over time. Mean DTI indices of the white matter skeleton plotted as a function of age, with two measure points for each participant plotted for the ADHD group (Left) and the non-ADHD group (Right).
Figure 5. Change in white matter microstructure plotted as in Figure 4, except that values are normalized with respect to t1.

Discussion

Here, we investigated whether developmental changes in white matter microstructure over adolescence and early adulthood differ between participants with and without ADHD. Furthermore, we investigated whether white matter development was related to the developmental changes in severity of ADHD symptoms. Therefore, we used a prospective longitudinal study with DTI scanning at two time points during adolescence. Overall, ADHD symptoms decreased over time. While ADHD symptoms of persistent and remittent ADHD groups were equal at baseline, by the second follow-up the remittent ADHD group showed significantly lower symptoms. However, at both time points both ADHD groups had more ADHD symptoms than the non-ADHD group. Despite the change in ADHD score over time, change in white matter microstructure was not found to be different between participants with and without ADHD. Furthermore, change in ADHD score was not found to be related to the change in white matter microstructure as measured using FA and MD indices.

The developmental change in ADHD symptoms reported here is in line with previous studies. These studies showed the complex interaction of various factors (e.g., comorbidities, familiarity, and age) on the remission rate, and most importantly the influence of the definition of remission (Biederman et al., 1996; Biederman et al., 2000). Biederman et al. (2000) reported more than 60 percent syndromatic remission (i.e., not meeting the full diagnostic criteria for ADHD) by the age of 18-20 years old. In the current
study, we report syndromatic remission percentages of 56% based on parent-rated Conners’ questionnaires.

Although the developmental change in ADHD symptoms was clearly observable, at the level of white matter microstructure developmental change was limited. More specifically, no developmental changes were observed in FA, whereas small changes mainly localized in frontal and limbic regions were observed in MD. These regions, and more specifically cortico-subcortical, fronto-temporal, and limbic tracts, are also reported in cross-sectional DTI studies investigating white matter development during and beyond adolescence (Asato, Terwilliger, Woo, & Luna, 2010; Tamnes et al., 2010). In addition, a longitudinal study showed ongoing development of cortico-limbic association tracts into adulthood, completed maturation of fronto-cortical and –subcortical tracts by the age of 16, and completed development of projection tracts by late childhood (Simmonds, Hallquist, Asato, & Luna, 2014). Importantly, that study points out that white matter development of prefrontal regions (and other late-maturing regions) does not follow a linear trajectory, but rather accelerated development during childhood reaching a plateau during adolescence and accelerating again during early adulthood. In our sample, developmental changes in white matter were not found to be dependent on age (see figure 4 and 5). One could attribute this to the size of the change in white matter microstructure in our sample in general, which was not significantly present in FA and rather small in MD.

Although the developmental change in ADHD symptoms was clearly observable and developmental changes were present in MD, we were unable to determine a relationship between change in ADHD symptoms and change in white matter microstructure. This indicates that the biological change in the white matter does not have a meaningful impact on the change in severity of ADHD symptoms over time. In other words, white matter might not be a suitable biomarker to characterize the development of ADHD over age. In line with the dimensional association findings, the developmental changes in white matter did not differ between categorical ADHD (persistent and/or remittent) and non-ADHD groups.

To the best of our knowledge, no previous studies have reported findings concerning the link between the developmental changes in ADHD pathology and developmental changes in white matter microstructure. Cross-sectional DTI studies investigating developmental changes in ADHD reported lower FA in tracts implicated during high-level as well as sensorimotor functions in ADHD (independent of current diagnosis) (Cortese et al., 2013). Of note, in this sample the mean follow-up age was 41 year old, whereas our sample, in contrast, had a mean follow-up age of 20 years old. Furthermore,
although this study used a 3T scanner, only 6 diffusion weighted directions were collected, limiting the precision of the assessment of the diffusion tensor. Another cross-sectional study reported abnormal white matter microstructure in tracts related to attentional control and hot cognitive-affective processes in persistent ADHD (Shaw et al., 2015). More specifically, effects of decreased FA were mostly driven by diffusion perpendicular to the axon (radial) and related to persistence of inattentive symptoms. In contrast to present study, this study did not use a voxelwise whole brain approach, but investigated 11 a priori selected tracts. Finally, in a previous cross-sectional study we showed that, across 101 participants with childhood ADHD, a decrease in hyperactive/impulsive symptoms was related to white matter microstructure of the corticospinal tract at follow-up (Francx et al., 2015). These participants belonged to the same cohort as participants described in present study, however, in present study a second MRI follow-up was added to the data. Using longitudinal MRI data (from t1 and t2), we could not replicate the findings revealed by cross-sectionally investigating the change from t0 to t1. A possible reason might be found in the developmental trajectory of white matter microstructure in the corticospinal tract of healthy participants. Previous studies in healthy participants showed that white matter develops more in childhood and reaches a plateau in early adulthood (Lebel & Beaulieu, 2011). With a mean age of 20 years old, our second follow-up might cover this age range.

This is the first longitudinal DTI study in an adolescent ADHD sample. Longitudinal studies have the advantage over cross-sectional studies that individual development can be followed, avoiding the extrapolation of inter-individual to intra-individual development. Furthermore, no retrospective recall of ADHD symptoms is necessary. Limitations of the current study are that there was some variation in informants and measures which is often inevitable in longitudinal studies that span a wide age range. However, we consistently used the Conners’ Parent-rated questionnaires obtained at each time point for our dimensional analyses. Though diagnostic interviews might be more accurate, at t1 the correlation between the Conners’ T scores and the K-SADS diagnostic interview was high ($r=0.7$). Secondly, a larger sample size might allow for more sensitivity for small developmental effects. Finally, in longitudinal studies the difference between developmental change and measurement error is difficult to assess. However, while the change in DTI indices over time was relatively small, we can assume that the measurement error was small too.

In conclusion, we did not observe a relation between developmental changes in ADHD symptoms and changes in white matter microstructure. As discussed, this might indicate that white matter microstructure is not a sensitive measure to assess the
neurobiological process that is related to changes of ADHD symptoms over time. The absence of this association needs to be interpreted with caution and our results should be replicated in another sample.
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