Cerebellar atypicalities in autism?

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Cerebellar atypicalities in autism?

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Running title: No cerebellar atypicalities in autism
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Abstract

**Background.** The cerebellum contains more than 50% of the brain neurons and is involved in social cognition. Cerebellar anatomical atypicalities have repeatedly been reported in individuals with autism. However, studies have yielded inconsistent findings, likely because of a lack of statistical power, and did not capture the clinical and neuroanatomical diversity of autism. Our aim was to better understand cerebellar anatomy and its diversity in autism.

**Methods.** We studied the cerebellar grey matter morphology in 274 individuals with autism and 219 controls of a multicenter European cohort (EU-AIMS LEAP). To ensure the robustness of our results, we conducted lobular parcellation of the cerebellum with two different pipelines in addition to voxel-based morphometry. We performed statistical analyses with linear, multivariate - including normative modeling - and a meta-analytic approach to capture the diversity of cerebellar anatomy in individuals with autism and controls. Last, we performed a dimensional analysis of cerebellar anatomy in an independent cohort of 352 individuals with autism-related symptoms.

**Results.** We did not find any significant difference in the cerebellum when comparing individuals with autism and controls using linear models. In addition, there were no significant deviations in our normative models in the cerebellum in individuals with autism. Finally, we found no evidence of cerebellar atypicalities related either to age, IQ, sex or social functioning in individuals with autism.

**Conclusions.** Despite positive results published in the last decade from relatively small samples, our results suggest that there is no striking difference in cerebellar anatomy of individuals with autism.

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Article body: 3974 words, 2 tables and 4 figures
Supplementary Material: 1 word document, including 13 figures and 19 tables
Introduction

The cerebellum contains more than 50% of the neurons of the brain (1), has almost 80% of the surface area of the neocortex (2) and is known to be involved in a broad range of cognitive functions including social cognition (3,4).

A large body of literature, including preclinical (5,6), histopathology, genetic and neuroimaging studies (see Fatemi et al. 2012 (7), Wang et al. 2014 (8) for a full review) has established the involvement of the cerebellar circuits in social cognition and the physiopathology of autism spectrum disorder (hereafter “autism”).

More than 40 prior studies reported anatomical atypicalities in the cerebellum in autism in relatively small samples. In a meta-analysis, Traut et al. (9) reported a significantly larger global cerebellar volume in individuals with autism compared to controls, though with a small effect size. Further, they did not replicate this finding in a large sample of 681 subjects from the Autism Brain Imaging Data Exchange database. Because the studies included in the meta-analysis were generally underpowered, the authors found that the number of significant findings was larger than expected. To date, despite many studies (9) investigating the cerebellar structure in autism, no consistent atypicalities have been found.

Several reasons may explain such discrepant findings. First, it may be that there really are no group differences in the cerebellum between individuals with autism and controls and that previous positive findings are the consequence of a publication bias from a large number of small underpowered studies (9). Second, there might be differences, but cerebellar morphological alterations in autism are subtle and located only in specific parts of the
cerebellum, such as the vermis or Crus I (5,10), which has not been investigated in large multicenter studies (9). Third, because autism is a heterogeneous condition (11–15), there might be distinct subgroups of individuals with autism with different pathophysiological mechanisms and different cerebellar morphological patterns that might be correlated with clinical dimensions such as sensory motor atypicalities (16,17). In that case, absent or discrepant findings might be related to the heterogeneity of subjects included in the studies, diluting consistent neural features across all subjects. Fourth, different segmentation methods could account for the variability in the results across studies - as different parcellations algorithms have been developed for the cerebellum with various outcomes. To the best of our knowledge, no study has investigated lobular cerebellar atypicalities in autism comparing different parcellation techniques. Last, there is a need to employ novel methods that can quantify individual deviations from a normative pattern without relying on group means, such as normative modelling approaches (14,16).

Our goal, therefore, was to study cerebellar anatomy of individuals with autism in a large multicenter sample, while taking into account these methodological and clinical considerations, in a large multicenter sample. First, we compared the cerebellar anatomy of individuals with autism and controls using three different standard approaches, at both lobular and voxel levels. Next, to move beyond standard case-control paradigms, we used normative modelling to quantify deviations from a normative pattern to best characterize sample heterogeneity. Finally, we studied how, within autism, cerebellar anatomy was associated with variation in clinical features.
Methods and Materials

Participants

We analyzed data from the EU-AIMS Longitudinal European Autism Project (LEAP) (18). This cohort is a large multicenter, observational study, which aims to identify and validate stratification biomarkers in autism. We included individuals from the LEAP cohort (individuals with autism and controls recruited in six centers), with a full-scale intellectual quotient (FSIQ) > 70.

Parcellation of cerebellar lobules

All participants were scanned with a 3T MRI scanner at 6 different sites, in Cambridge, UK (Siemens Verio), London, UK (General Electrics Discovery mr750), Mannheim, Germany (Siemens TimTrio), Nijmegen, Netherlands (Siemens Skyra), Rome, Italy (GE, Signa HDxt) and Utrecht (Phillips Medical System, Achieva / Ingenia CX). High-resolution structural T1-weighted volumetric images were acquired with full head coverage, at 1.2-mm thickness with 1.2x1.2-mm in-plane resolution. Acquisition protocols are reported in Supplementary Table S1. A flow chart describing the quality control steps (Supplementary Figure S1), a description of the motion-related artifacts (Supplementary Figure S2), the most frequent parcellation errors (Supplementary Figure S3) and an example of parcellation defects related to a cyst of the posterior fossa (Supplementary Figure S4) are reported in the supplementary material section. A comparison of the subjects included and excluded from the analyses is reported in Supplementary Figure S5. There was no difference in the proportion of individuals with autism/controls in the excluded/included subjects, however FSIQ and age were lower in the participants excluded from the analyses.
We used two different methods to perform parcellation at a lobular level (Figure 1A, 1B and Supplementary Figure S5). First, we used the CERES pipeline (19) (Figure 1B and Supplementary Figure S5). This pipeline relies on an atlas that has been compared to manual tracing (26). Second, we used the SUIT pipeline (20) (see Supplementary Methods). Although the SUIT pipeline has not been compared to manual tracing, this toolbox provides a segmentation of the cerebellar vermis, a region previously linked to autism (10,21–23). We performed a careful visual quality check (Supplementary Methods) and compared the outcome of both parcellation measures. The description of the parcellation outcomes is reported in Supplementary Methods.

**Voxel based morphometry analysis (VBM)**

We used VBM SUIT procedure (20) to look at finer-grained, voxel-level differences that parcellation-based approaches might not provide. This method, designed for the cerebellum, relies on a normalization to a probabilistic atlas of the cerebellar lobules in the anatomical space and is further described in Supplementary Methods.

**Clinical features**

The full clinical assessment of the EU-AIMS cohort is described elsewhere (24) and in Supplementary Methods.

To assess if the clinical dimensions of autism were correlated with the cerebellar morphology while limiting the number of multiple comparisons, we selected three clinical variables based on the literature. First, we selected the social responsiveness score (SRS-2, 2nd version, T-score) in order to see how the cerebellar structure was correlated to the severity of autism (25). Second, we selected the sensory atypicalities, based on the Total Short Sensory Profile scale (26) in the EU-AIMS sample (17). Last, we selected the ADHD DSM-IV rating scale to
measure severity of ADHD symptoms (Supplementary Methods), a frequent comorbidity of autism. Atypicalities of the cerebellum (notably in the vermis of the cerebellum) have been reported in ADHD (27–29). These results suggested that cerebellar atypicalities reported in autism might be at least partly related to comorbid ADHD symptoms. In addition, we performed secondary analyses to assess the association between ADOS Calibrated Severity Score ((30) - ADOS-CCS), the Restricted Behavior Scale Revised ((31) - RBS-R) and cerebellar structure.

**Normative modelling**

This novel method has been described in detail elsewhere (16) and successfully applied to mental disorders and autism in particular (12,14). Structural T1-weighted images were pre-processed with the SUIT pipeline (see VBM section) which is specifically adapted to the cerebellum (20). A Gaussian process regression model was trained at each voxel in the control cohort using age, sex, site of inclusion, FSIQ and intracranial volume (ICV) as covariates to predict cerebellar grey matter volume. We ran a secondary analysis, while excluding FSIQ of the covariates. We generated normative probability maps, which quantify the deviation of each participant from the normative model for cerebellar gray matter volume at each voxel. These maps were then compared in patients and controls (see a description of our normative model in Supplementary Methods). To evaluate the goodness of fit of our normative model in the neurotypical subjects, we computed a Rho map of the cerebellum showing the correlation between the predicted and the actual values in the cerebellum. In addition, we calculated the symmetric mean square error (SMSE) of our model in the neurotypical subjects.
Supervised learning

We conducted classification analyses to predict which individuals belonged to the autism / controls groups based on the cerebellar anatomy (Supplementary Methods).

Meta-analytical approach and homogeneity measure

To study the variability across the different sites, we repeated our analyses in each site of inclusion. We compared the volume of each cerebellar sub-region with linear models, considering age, sex, ICV and FSIQ as covariates. Next, we conducted a meta-analysis across the sites of inclusion and estimated the q and the I2 statistics to study the heterogeneity of our results.

Statistical analyses

To decide how to consider linear covariates (age and FSIQ) in our model, we tested the best model fit in each site of inclusion between a linear, cubic or quadratic model (32). Details on the statistical analyses are reported in Supplementary Methods. For parcellation analyses, we conducted linear models, using scanning site, sex, age, FSIQ and ICV as covariates. We conducted Pearson’s correlations test to assess the correlation between volumetric measures of CERES and SUIT pipelines.

Statistical analyses of VBM are described in Supplementary Methods.

To test if clinical features were associated with the cerebellar structure, we conducted linear models only in the group of individuals with autism and regressed out the effect of age, sex, ICV, FSIQ and MRI scanning site.

We conducted heterogeneity focused analyses to understand the influence of age, sex and IQ in our statistical models. We compared two models: the first one including the variable of interest (age, sex or IQ) and its interaction with diagnosis, and the second not including the
variable of interest and the interaction term, as described in Bedford et al. (32) (Supplementary Methods). Last, we studied the effect of diagnosis on cerebellar structure only in males, only in females and in IQ and age-centered intervals (Supplementary Methods).

We performed an analysis in matched individuals with autism and controls (see Supplementary Methods) to ensure that the results from the main analyses were not driven by demographic differences between individuals with autism and controls.

*Dimensional analyses in a transdiagnostic pediatric cohort*

We conducted analyses in the Healthy Brain Network (33), an independent transdiagnostic mental health pediatric cohort recruited in New-York, USA. In this more heterogenous population of individuals with autism-related symptoms without a formal diagnosis of autism, we studied the influence of the clinical dimensions of autism using the SRS-2 T-score and two of its subscales, measuring social / communication impairments or repetitive and restrictive behaviors. A complete description of the study population and its clinical characteristics is reported in Supplementary Methods. We selected individuals with FSIQ > 70 and symptoms related to autism, as defined by SRS-2 T-score > 60. We defined both a “relaxed” (n = 352, SRS-2 T-score > 60) and a “stringent” (n = 79, SRS-2 T-score > 76) sample.
Results

Population of the study

Demographics of the study population are reported in Table 1. There was no significant difference in age between patients and controls; however, there were more males than females in patients compared with controls and a higher FSIQ in controls compared to patients. Thus, FSIQ and sex were included as covariates in our statistical analyses.

Comparison of CERES and SUIT pipeline.

We performed cerebellar parcellation using SUIT and CERES pipeline and compared parcellation outcomes of both pipelines (Supplementary Methods). We found a strong positive relationship in Crus I \((r = 0.72 \; p < 0.001)\), the anterior lobe of the cerebellum \((r = 0.73 \; p < 0.001)\), the postero-inferior lobe of the cerebellum \((r = 0.75 \; p < 0.001)\) between SUIT and CERES except for Crus II \((r = 0.55 \; p\text{-value} < 0.001)\) where we found a moderate positive relationship (Supplementary Figure S6).

Case-control analyses.

   Effect of age and FSIQ. We found that modelling age and FSIQ with a linear effect was more accurate, as opposed to a cubic or quadratic effect (Supplementary Results and Figure S7).

   Parcellation and VBM. We did not find any significant effect of autism diagnosis in our regions of interest using the CERES or SUIT pipeline. Effect of the diagnosis of autism (CERES pipeline) on cerebellar volumes are reported in Table 2 and Supplementary Figure S8.
There was no effect of autism diagnosis in the cerebellar vermis or lobule VI-VII part of the vermis (Supplementary Table S3), a region previously involved in autism. When conducting the analyses at the voxel level (VBM - SUIT pipeline, Supplementary Methods), we did not find any effect of diagnosis or any significant sex by diagnosis interaction, which was consistent with our findings using a Region-of-Interest approach.

**Heterogeneity of cerebellar anatomy in autism**

We found no evidence of cerebellar atypicalities in autism related either to sex, age, FSIQ or the clinical features of autism. We tested in analyses focused on heterogeneity the effects of sex, age and FSIQ on cerebellar anatomy. We found no evidence for a strong heterogeneity related to these variables in our linear models (see Supplementary Results and Figure S9). In addition, we found no significant sex by diagnosis interaction for any of the cerebellar regions. Also, when restricting analyses to either males or females, results did not change (Supplementary Tables S4). Regarding age and FSIQ, there were no significant (i) age by diagnosis interaction or (ii) effect of diagnosis on cerebellar structure in age-centered intervals, (iii) diagnosis by FSIQ interaction or (iv) effect of diagnosis on cerebellar structure in FSIQ-centered intervals (see Supplementary Results and Figure S10). Repeating our analyses in a sample of individuals with autism matched with controls based on sex, age and FSIQ did not change our results (Supplementary Results and Tables S5A to S5C). Within the autism sample, there were no significant correlations between (i) SRS-2 T-score (Supplementary Figure S11), (ii) diagnosis of ADHD or (iii) sensory alterations and cerebellar structure. Similarly, there were no associations between (i) restricted and repetitive behaviors (Supplementary Figure S12) or (ii) ADOS CCS score (Supplementary Figure S13) and cerebellar structure.
In the Healthy Brain Network cohort, we found no association between (i) total SRS-2 T-score, (ii) SRS-SCI-2 T-score (communication/interaction score), (iii) SRS-2 RRB T-score (restrictive and repetitive behaviors) and cerebellar structure, nor SRS-2 T-score by FS IQ and SRS-2 T-score by age interaction in both the “relaxed” and the “stringent” samples (see Supplementary Results and Tables S6).

**Multivariate analyses**

**Normative Modelling.** Despite a good fit of our normative model (Figure 2), we found no increased or decreased deviation in the cerebellar lobules (Figure 3 and Supplementary Table S7), when comparing individuals with autism to neurotypical control. There was no significant difference either at the whole cerebellum level or at a lobular level. Removing FS IQ from our model did not change our results.

**Support vector machine to predict diagnosis.** Our model did not predict the diagnostic category (individual with autism vs controls) above the level of chance, when considering as predictive features either regions of interest extracted from the cerebellar parcellation or VBM cerebellar maps. Balanced accuracy did not exceed 52% (Supplementary Table S8).

**Meta-analytical approach and site by site analysis**

We compared cerebellar anatomy in individuals with autism and controls in each site of inclusion (see Supplementary Table S9 for a description of clinical features across each site of inclusion and Figure 4 for the results of the meta-analysis). Using the CERES parcellation, two regions of interest were different in two sites: compared to controls there was an increase in the volume of the lobule VI in individuals with autism recruited in Rome (p = 0.035,
uncorrected) and an increase in the volume of Crus II (p = 0.048, uncorrected) in individuals with autism recruited in Cambridge. Using the SUIT parcellation, we found only a reduced volume of the vermis in individuals with autism compared to controls in Nijmegen (p = 0.041, uncorrected).

It is important to note that the significant results obtained in two sites with the CERES pipeline were not significant using the SUIT pipeline (despite a good correlation between both measures reported in Supplementary Figure S6). For all significant results, the confidence interval (Figure 4) was large compared to the full sample analysis and close from 0. The meta-analysis metrics (q statistics and I2, see Supplementary Figure S10) suggested that there was no strong heterogeneity in the results across sites.
Discussion

A broad range of atypicalities in the anatomy of different cerebellar regions has been inconsistently reported in autism in the last decades, mostly in small sample size studies. Our goal was to study the cerebellar anatomy in a large harmonized multicentric cohort to reconcile previous results from the literature. We combined complementary statistical (both ‘traditional’ group case control paradigms and individual deviations using normative modelling, supervised learning) and neuroimaging (parcellation, VBM adapted to the cerebellum) methods to fully understand the cerebellar anatomy in autism.

We found that regardless of the analytical technique we employed, there was no case-control difference in the cerebellar anatomy. In addition, within autism, there was no correlation between cerebellar anatomy and clinical features. We discuss these results in the context of neuroimaging studies of autism and replicability / reproducibility issues in neuroimaging.

Like many neurodevelopmental and psychiatric disorders, autism is clinically heterogeneous and conceptualized as a spectrum rather than a sharply delineated condition. In cortical regions, there have been recent attempts to identify subgroups of individuals with autism using neuroanatomical features (34,35). However, to date, reports from MRI studies on cerebellar anatomy in autism are based on “traditional” case-control analysis, and mostly from relatively small samples. These studies typically reported cerebellar alterations in the Crus I region (5,29), in the anterior lobe (10) or in the vermis (21,22,36), which were correlated to clinical dimensions of autism. A meta-analysis on 30 studies on cerebellar anatomy in autism (9) reported a weak but significant association between autism diagnosis and increased global (overall) cerebellar volume (p = 0.049, uncorrected). In addition, this meta-analysis (9) studied the cerebellar volume in a larger sample (ABIDE dataset) but did not conduct a parcellation
analysis and studied the global volume of the cerebellum. However, the cerebellar cortex can be divided between an anterior part - connected to the sensory motor cortex - and a posterior / cognitive part - connected to the associative cortex. Because of this functional topography, it is critical to study the anatomy of the cerebellum at a lobular level.

We did not find a difference in terms of cerebellar sub-volume in individuals with autism compared to neurotypical controls. These results were consistent across two different parcellations methods and a voxel wise analysis. Parcellations were visually inspected by an expert rater blind of the diagnosis. These results are consistent with the meta-analysis that reported inconclusive results at a lobular level (9). Thus, we believe that there is no consistent difference in cerebellar morphology when using a classic case-control approach.

The discrepancy of previous results in the literature could be explained by different neuroimaging methods that they employed (10,37,38). Cerebellar parcellation can be performed manually, semi-automatically and fully automatically. Because of the heterogeneity of autism, it is critical to investigate its neuroanatomy in large multicenter samples to avoid false positive results (39). Also, manual and semi-automated segmentation methods are difficult to apply to large samples and there is a need to develop fully automated segmentation algorithms. However, fully automated parcellation methods rely on different atlases (20,40). To the best of our knowledge, our study is the first to compare different parcellation algorithms in a clinical population of individuals with autism.

We found a moderate to strong positive relationship between CERES (19) and SUIT (20). It is important to note that the definition of the lobules differs between both techniques, which rely on different atlases. The CERES pipeline relies on the atlas of Park et al. (40) where the vermis is merged into the cerebellar hemisphere. Thus, the Crus II region - where we only found a moderate correlation between both methods - encompasses part of the vermis in the CERES
pipeline as compared to the SUIT pipeline where the vermis is isolated from the hemisphere. This difference of definition in the cerebellar parcellation might partly explain the discrepant findings from previous studies. In our study, we analyzed the cerebellar volumetry with both techniques to ensure the robustness of our results and in both cases, we did not find differences between autism and neurotypical controls. In addition, we also used the SUIT pipeline (20) to perform analyses at a voxel level.

Our study has several strengths. Most of the prior studies investigating the anatomy of individuals with autism focused on the entire brain and did not investigate the cerebellum specifically. Because of the position of the cerebellum (distinct from the neo-cortex, in the posterior fossa) and its specific anatomical structure (high degree of folding), the analysis of the cerebellum requires specific tools and parcellation algorithms. In this paper, an expert rater, blind to diagnosis, visually inspected all cerebellar parcellations. To ensure the robustness of our results, we used different parcellation methods and statistical analyses to fully understand how the cerebellar structure might differ in individuals with autism and controls. We believe that to date, this is the most exhaustive study investigating the structural anatomy of the cerebellum in autism.

Several reasons could explain our negative results. One possibility might be lack of statistical power. However, all previous results on cerebellar anatomy included smaller samples (10), see also meta-analysis (9) suggesting that, if present, atypicalities could have been detected. In addition, cerebellar atypicalities have been repeatedly reported in other brain disorders such as schizophrenia in samples of the same size as this study (41,42). Heterogeneity in individuals with autism could also have explained our negative results in the case-control analyses. In that case, only a subgroup of individuals with specific pattern of
symptoms, intellectual functioning or age would display cerebellar atypicalities, which might be missed with classical group comparisons. To fully explore this hypothesis, we conducted a wide range of analyses to investigate the effect of sex, age, IQ, social functioning, sensory atypicalities, diagnosis of ADHD, repetitive and restrictive behaviors. We found no evidence of subgroup specific atypicalities of the cerebellum. In an independent cohort of individuals with autism-related symptoms, with higher heterogeneity compared to the EU-AIMS sample, the severity of autistic symptoms had no influence on cerebellar structure. Last, we also conducted a normative model analysis to investigate differences at the individual level. However, we detected no significant positive or negative deviations from the norm despite a good fit of our model. Although this approach has been successfully applied to autism with positive results in the cerebral cortex (12), our results were negative in the cerebellum when using a similar sample.

Our meta-analytical approach revealed marginally significant results (Figure 4). These results were not replicated when using a different parcellation method. This suggests that interpreting results in small samples is not relevant and leads to inconsistent results that are sensitive to parcellation methods. This was the case of the studies published to date on cerebellar parcellation (including a study published by our group (10)). These results explain how false positive results might arise from the literature with real-life data.

Several limitations should be considered before interpreting our results. Concerns have been raised regarding the validity of psychometric properties of the Short Sensory Profile scale (26). While our paper is focused on cerebellar volumetry using 3T MRI, this approach has limitations. The cerebellum is a highly folded structure with almost 80% of the surface area of the neocortex. Partial volume issues are thus more prominent for the cerebellum. Thus, 7T MRI
(2) might be more able to detect atypicalities in cerebellar anatomy of individuals with autism. Last, it is possible that although cerebellar anatomy appears normal using our approaches there may still be differences in functional and structural connectivity. This is the focus of future work.

To the best of our knowledge, this is the largest study to investigate the anatomy of the cerebellum in autism. Our results strongly suggest that there is no significant difference in cerebellar anatomy between individuals with autism and controls. In the context of replicability and reproducibility issues in science, our paper underlines the interest of using different statistical / neuroimaging methods and a large sample to address the same research question and avoid inconsistent results.
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Disclosure

Dr. Tillmann has served as a consultant for Hoffmann–La Roche. Dr. Baron-Cohen has served as an author, consultant, or lecturer for Ability Partner, Clarion Healthcare, Expo Medica, Eli Lilly, GLGroup, Kompetento, Medice, Prima Psychiatry, Prophase, Roche, Shire, and System Analytic; he receives royalties for textbooks and diagnostic tools from Huber/Hogrefe, Kohlhammer, and UTB. Dr. Charman has served as a consultant for F. Hoffmann–La Roche and Servier; and he has received royalties from Guilford Publications and Sage Publications. Dr. Beckmann is co-founder of SBGneuro. Dr. Buitelaar has served as a consultant, advisory board member, and/or speaker for Angelini, Janssen Cilag BV, Novartis, Medice, Roche, Servier, and Takeda/Shire. Dr. Murphy has received honoraria from Roche and Servier, and he has received grant support from the Medical Research Council (UK), the National Institute for Health Research, and Horizon 2020 and the Innovative Medicines Initiative (European Commission). The other authors report no biomedical financial interests or potential conflicts of interest.
### Tables

**Table 1: Study population**

<table>
<thead>
<tr>
<th></th>
<th>ASD n = 274</th>
<th>Controls n = 219</th>
<th>Statistics</th>
<th>P value</th>
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<tr>
<td><strong>Mean Age (SD) [min - max]</strong></td>
<td>17 (5) [7.00 - 30]</td>
<td>17 (5) [6 - 30]</td>
<td>t-test</td>
<td>NS.</td>
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<tr>
<td><strong>Sex ratio (% of Males)</strong></td>
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<td>0.64</td>
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<tr>
<td><strong>Mean full scale IQ (SD) [min - max]</strong></td>
<td>103 (19) [70 - 148]</td>
<td>108 (18) [70 - 142]</td>
<td>t-test</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td><strong>Mean verbal IQ (SD) [min - max]</strong></td>
<td>102 (20) [70 - 160]</td>
<td>104 (18) [70 - 158]</td>
<td>t-test</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td><strong>Mean performance IQ (SD) [min - max]</strong></td>
<td>104 (19) [70 - 150]</td>
<td>105 (20) [70 - 147]</td>
<td>t-test</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td><strong>Mean ADOS 2 (SD) [min - max]</strong></td>
<td>Social-affect: 6 (2) [1 - 10] Communication: 4 (2) [1 - 10] CSS total: 4 (3) [1 - 10]</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Mean ADI-R (SD) [min - max]</strong></td>
<td>Social-affect: 16 (6) [1 - 28] Communication: 12 (5) [0 - 25] RRB: 4 (2) [0 - 12]</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Mean SRS-2 T-score (SD) [min - max]</strong></td>
<td>70 (12) [43 - 90]</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Diagnosis of ADHD (yes / no)</strong></td>
<td>98 / 144</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Mean RBS-R score (SD) [min - max]</strong></td>
<td>15 (13) [0 - 73]</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Mean SSP score (SD) [min – max]</strong></td>
<td>130 (36) [4 – 189]</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

ASD: individuals with autism spectrum disorders
NS: non significant ; SRS-2 T-score: social responsiveness scale, 2nd version, T-score ; RBS-R: restricted and repetitive behaviors – revised scale; SSP: Short Sensory Profile scale; ADOS-CSS: ADOS Calibrated Severity Score ; ADI-R: Autism Diagnostic Interview - Revised ; ADOS 2: Autism Diagnostic Observation Schedule ; Sites of inclusion : C: Cambridge, K: King’s College London, M: Mannheim, N: Nijmegen ; R: Rome, U: Utrecht, a: data missing for 50 individuals, b: data missing for 32 individuals, c: data missing for 56 individuals, d: data missing for 59 individuals
Table 2: No association of autism with cerebellar parcellation - CERES analysis

<table>
<thead>
<tr>
<th>ROI</th>
<th>p-value</th>
<th>t-value</th>
<th>CI [inf ; sup]</th>
<th>dof</th>
<th>Cohen’s $f^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellum</td>
<td>0.3824</td>
<td>-0.87</td>
<td>[-2.26 ; 0.87]</td>
<td>10</td>
<td>0.002</td>
</tr>
<tr>
<td>Ant. lobe</td>
<td>0.8042</td>
<td>0.25</td>
<td>[-0.24 ; 0.31]</td>
<td>10</td>
<td>0.024</td>
</tr>
<tr>
<td>Lobule VI</td>
<td>0.3276</td>
<td>-0.98</td>
<td>[-0.59 ; 0.20]</td>
<td>10</td>
<td>0.024</td>
</tr>
<tr>
<td>Crus I</td>
<td>0.3076</td>
<td>-1.02</td>
<td>[-0.91 ; 0.29]</td>
<td>10</td>
<td>0.007</td>
</tr>
<tr>
<td>Crus II</td>
<td>0.3616</td>
<td>-0.91</td>
<td>[-0.59 ; 0.21]</td>
<td>10</td>
<td>0.002</td>
</tr>
<tr>
<td>Lobule VIIb</td>
<td>0.3627</td>
<td>-0.91</td>
<td>[-0.32 ; 0.12]</td>
<td>10</td>
<td>0.006</td>
</tr>
<tr>
<td>Post. Inf. lobe</td>
<td>0.8685</td>
<td>0.17</td>
<td>[-0.51 ; 0.61]</td>
<td>10</td>
<td>0.016</td>
</tr>
</tbody>
</table>

CI: confidence interval, inf: inferior, sup: superior; dof: degrees of freedom, Ant. lobe: anterior lobe of the cerebellum, Post. Inf. Lobe: postero-inferior lobe of the cerebellum; diagnosis (group): individuals with autism or controls; sex, age, site of inclusion, ICV and FSIQ were included as covariates.
Figures

Figure 1: Cerebellar parcellation

Legend:
Panel A: Visualization of the cerebellum with cerebellum-value-map package https://gitlab.com/shan-utils/cerebellum-value-map (52) Anterior cerebellum (Lobules I - V) = green; Lobule VI = light blue; Crus I and Crus II = yellow; Lobule VIIb = orange; Postero-inferior lobe (red); Vermal portion of lobules VI and VII (dark blue); Vermal portion of postero-inferior lobe (white).
Panel B: Cerebellar parcellation and intracranial volume measured with the CERES pipeline. Top panel = Intracranial volume; Middle panel = tissue classification; Lower panel = cerebellar parcellation.

Figure 2: Goodness of fit of the normative model in controls

Legend:
SMSE: symmetric mean square error, evaluating the goodness of fit of the model.
Rho map showing the correlation between the predicted and the actual value for each voxel of the cerebellum. The relationship between the covariates (age, sex, ICV, full scale IQ, and site of inclusion) and cerebellar anatomy is very strong with nearly always positive correlation and SMSE < 1.

Figure 3: No difference of positive / negative deviations in the regions of interest of the cerebellum in autism vs neurotypical populations

Legend:
Right Ant. Lobe: right anterior lobe of the cerebellum
Left Ant. Lobe: left anterior lobe of the cerebellum
ASD: individuals with Autism Spectrum Disorders
TD: typically developing individuals

Figure 4: Cerebellar parcellation in each site of inclusion in individuals with autism and typically developing controls

Legend:
Panel A. Effect of diagnosis (95% confidence intervals) for the total grey matter volume of the cerebellum and each subregion of the cerebellum (CERES pipeline), in the whole sample (dark red) and each site of inclusion (dark blue).
Panel B. Effect of diagnosis (95% confidence intervals) for the cerebellar vermis and the vermal portion of lobule VI / VII (SUIT pipeline), in the whole sample (dark red) and each site of inclusion (dark blue).
ASD: individuals with Autism Spectrum Disorders; TD: Typically developing individuals
* : uncorrected p-value = 0.035; ** : uncorrected p-value = 0.048; + : uncorrected p-value = 0.041
Y axis: regions of the cerebellum
X axis: residuals of linear models, including age, sex, intracranial volume and full-scale IQ for each site of inclusion (dark blue) and age, sex, intracranial volume, full-scale IQ and site of inclusion (dark red) for the whole sample.
Pi: postero-inferior cerebellum; Lob: lobule; ant. cereb: anterior lobe of the cerebellum.
References

3578–3588.
A  Schematic of the cerebellar parcellation depicted in a cerebellar flat map

B  Parcellation of the cerebellum with the CERES pipeline
Histogram of SMSE neurotypical subjects

Rho map in neurotypical subjects