

Cerebellar Atypicalities in Autism?

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ABSTRACT

BACKGROUND: The cerebellum contains more than 50% of the brain's 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 cerebellar gray matter morphology in 274 individuals with autism and 219 control subjects of a multicenter European cohort, EU-AIMS LEAP (European Autism Interventions–A Multicentre Study for Developing New Medications; Longitudinal European Autism Project). To ensure the robustness of our results, we conducted lobular parcellation of the cerebellum with 2 different pipelines in addition to voxel-based morphometry. We performed statistical analyses with linear, multivariate (including normative modeling), and meta-analytic approaches to capture the diversity of cerebellar anatomy in individuals with autism and control subjects. Finally, 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 control subjects 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 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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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 (7,8) 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 with control subjects, although 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 (ABIDE) 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 control subjects 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 have 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

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segmentation methods could account for the variability in the results across studies because different parcellations algorithms have been developed for the cerebellum with various outcomes. To our knowledge, no study has investigated lobular cerebellar atypicalities in autism comparing different parcellation techniques. Finally, there is a need to use novel methods that can quantify individual deviations from a normative pattern without relying on group means, such as normative modeling 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. First, we compared the cerebellar anatomy of individuals with autism and control subjects using three different standard approaches at both lobular and voxel levels. Next, to move beyond standard case-control paradigms, we used normative modeling to quantify deviations from a normative pattern to best characterize sample heterogeneity. Finally, we studied how cerebellar anatomy was associated with variation in clinical features within autism.

METHODS AND MATERIALS

Participants

We analyzed data from the EU-AIMS 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 control subjects recruited in 6 centers) with a Full Scale IQ (FSIQ) > 70.

Parcellation of Cerebellar Lobules

All participants were scanned with a 3T magnetic resonance imaging (MRI) scanner at 6 different sites: Cambridge, United Kingdom (Siemens Verio); London, United Kingdom (General Electric Discovery mr750); Mannheim, Germany (Siemens TimTrio); Nijmegen, the Netherlands (Siemens Skyra); Rome, Italy (GE, Signa HDxt); and Utrecht, the Netherlands (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 1.2×1.2 mm in-plane resolution. Acquisition protocols are reported in Table S1. A flow chart describing the quality control steps (Figure S1), a description of the motion-related artifacts (Figure S2), the most frequent parcellation errors (Figure S3), and an example of parcellation defects related to a cyst of the posterior fossa (Figure S4) are reported in the Supplement. A comparison of the subjects included and excluded from the analyses is reported in Figure S5. There was no difference in the proportion of individuals with autism/control subjects in the excluded/included subjects; however, FSIQ and age were lower in the participants excluded from the analyses.

We used 2 different methods to perform parcellation at a lobular level (Figure 1A, B; Figure S5). First, we used the CERES pipeline (19) (Figure 1B; Figure S5). This pipeline relies on an atlas that has been compared with manual tracing (20). Second, we used the SUIT pipeline (21) (see Supplemental Methods). Although the SUIT pipeline has not been compared with manual tracing, this toolbox provides a

segmentation of the cerebellar vermis, a region previously linked to autism (10,22–24). We performed a careful visual quality check (Supplemental Methods) and compared the outcome of both parcellation measures. The description of the parcellation outcomes is reported in Supplemental Methods.

Voxel-Based Morphometry Analysis

We used a voxel-based morphometry (VBM) SUIT procedure (21) to look at finer-grained, voxel-level differences that parcellation-based approaches might not provide. This method, designed for the cerebellum, relies on normalization to a probabilistic atlas of the cerebellar lobules in the anatomical space and is further described in Supplemental Methods.

Clinical Features

The full clinical assessment of the EU-AIMS cohort is described elsewhere (25) and in Supplemental Methods.

To assess if the clinical dimensions of autism were correlated with 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 from the Social Responsiveness Scale, Second Edition (SRS-2) (T score) to see how the cerebellar structure was correlated to the severity of autism (26). Second, we selected sensory atypicalities based on the Total Short Sensory Profile scale (27) in the EU-AIMS sample (17). Finally, we selected the attention-deficit/hyperactivity disorder (ADHD) DSM-IV rating scale to measure severity of ADHD symptoms (Supplemental Methods), a frequent comorbidity of autism. Atypicalities of the cerebellum (notably in the vermis of the cerebellum) have been reported in ADHD (28–30). 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 the Autism Diagnostic Observation Schedule calibrated severity score (31), the Restricted Behavior Scale-Revised (32), and cerebellar structure.

Normative Modeling

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 preprocessed with the SUIT pipeline (see Voxel-Based Morphometry Analysis), which is specifically adapted to the cerebellum (21). 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 gray matter volume. We ran a secondary analysis excluding FSIQ from 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 control subjects (see a description of our normative model in Supplemental Methods). To evaluate the goodness-of-fit of our normative model in 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 symmetrical mean square error (SMSE) of our model in the neurotypical subjects.

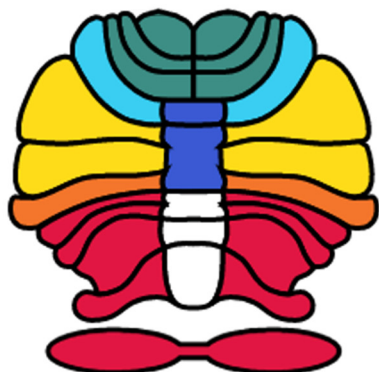
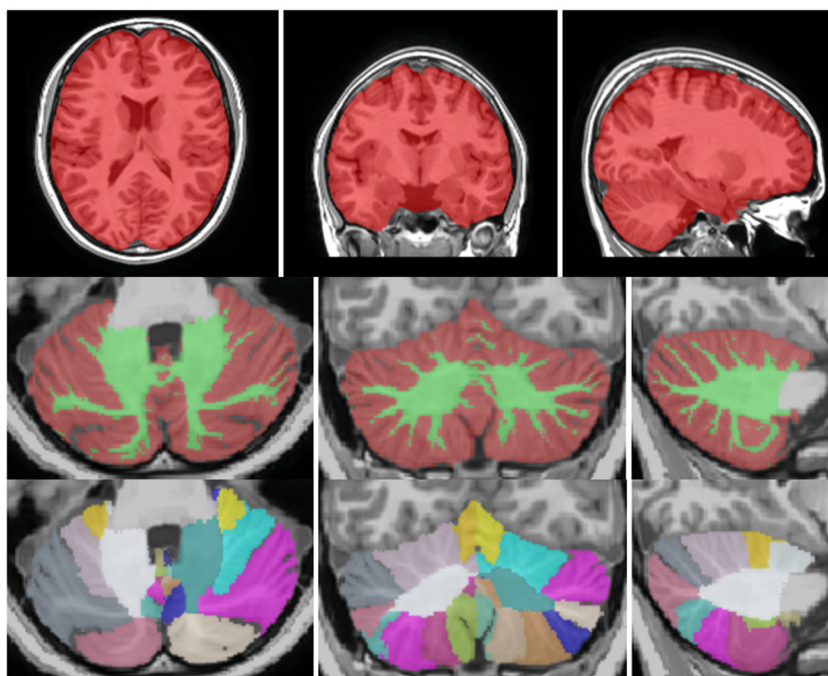
A Schematic of the cerebellar parcellation depicted in a cerebellar flat map**B** Parcellation of the cerebellum with the CERES pipeline

Figure 1. Cerebellar parcellation. **(A)** Visualization of the cerebellum with cerebellum-value-map package (<https://gitlab.com/shan-utis/cerebellum-value-map>) (42). Anterior cerebellum (lobules I–V) = green; lobule VI = light blue; Crus I and Crus II = yellow; lobule VIIb = orange; posteroinferior lobe = red; vermal portion of lobules VI and VII = dark blue; vermal portion of posteroinferior lobe = white. **(B)** Cerebellar parcellation and intracranial volume measured with the CERES pipeline. Top panel shows intracranial volume; middle panel shows tissue classification; lower panel shows cerebellar parcellation.

Supervised Learning

We conducted classification analyses to predict which individuals belonged to the autism and control groups based on cerebellar anatomy (Supplemental Methods).

Meta-analytic Approach and Homogeneity Measure

To study variability across the different sites, we repeated our analyses for each site of inclusion. We compared the volume of each cerebellar subregion with linear models, considering age, sex, ICV, and FSIQ as covariates. Next, we conducted a meta-analysis across the sites of inclusion and estimated q and I^2 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 (33). Details on the statistical analyses are reported in Supplemental 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 correlation between volumetric measures of the CERES and SUIT pipelines.

Statistical analyses of VBM are described in Supplemental 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 2 models, the first 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.* (33) (Supplemental Methods). Finally, we studied the effect of diagnosis on cerebellar structure only in males, only in females, and in IQ- and age-centered intervals (Supplemental Methods).

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

Dimensional Analyses in a Transdiagnostic Pediatric Cohort

We conducted analyses in the Healthy Brain Network (34), an independent transdiagnostic mental health pediatric cohort recruited in New York, New York. In this more heterogeneous 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 2 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 Supplemental 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 control subjects; however, there were more male than female patients compared with control subjects and a higher FSIQ in control subjects than in patients. Thus, FSIQ and sex were included as covariates in our statistical analyses.

Comparison of CERES and SUIT Pipelines

We performed cerebellar parcellation using the SUIT and CERES pipelines and compared parcellation outcomes of both pipelines (Supplemental Methods). We found a strong positive relationship in crus I ($r = 0.72$; $p < .001$), the anterior lobe of the cerebellum ($r = 0.73$; $p < .001$), and the posteroinferior lobe of the cerebellum ($r = 0.75$; $p < .001$) between SUIT and CERES, and for crus II ($r = 0.55$; $p < .001$), we found a moderate positive relationship (Figure S6).

Case-Control Analyses

Effect of Age and FSIQ. We found that modeling age and FSIQ with a linear effect was more accurate, as opposed to a cubic or quadratic effect (Supplemental 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 pipelines. Effects of the diagnosis of autism (CERES pipeline) on cerebellar volumes are reported in Table 2 and Figure S8. There was no effect of autism diagnosis in the cerebellar vermis or lobule VI/VII part of the vermis (Table S3), a region previously involved in autism. When conducting the analyses at the voxel level (VBM–SUIT pipeline, Supplemental 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 to sex, age, FSIQ, or the clinical features of autism. In analyses focused on heterogeneity, we tested 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 (Supplemental Results and Figure S9). In addition, we found no significant sex by diagnosis interaction for any of the cerebellar regions. When restricting analyses to either males or females, results did not change (Table S4). Regarding age and FSIQ, there was no significant age by diagnosis interaction, effect of diagnosis on cerebellar structure in age-centered intervals, diagnosis by FSIQ interaction, or effect of diagnosis on cerebellar structure in FSIQ-centered intervals (Supplemental Results and Figure S10). Repeating our analyses in a sample of individuals with autism matched with control subjects based on sex, age, and FSIQ did not change our results (Supplemental Results and Table S5A–C). Within the autism sample, there were no significant correlations between SRS-2 T score (Figure S11), diagnosis of ADHD, or sensory alterations and cerebellar structure. Similarly, there were no associations between restricted and repetitive behaviors (Figure S12) or Autism Diagnostic Observation Schedule calibrated severity score (Figure S13) and cerebellar structure.

In the Healthy Brain Network cohort, we found no association between total SRS-2 T score, SRS-2 communication/interaction T score, or SRS-2 restrictive and repetitive behaviors T score and cerebellar structure or between SRS-2 T score by FSIQ interaction and SRS-2 T score by age interaction in both the relaxed and the stringent samples (Supplemental Results and Table S6).

Multivariate Analyses

Normative Modeling. Despite a good fit of our normative model (Figure 2), we found no increased or decreased deviation in the cerebellar lobules (Figure 3 and Table S7) when comparing individuals with autism to neurotypical control subjects. There was no significant difference either at the whole cerebellum level or at a lobular level. Removing FSIQ 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. control subject) above the level of chance, when considering either regions of interest extracted from the

Table 1. Study Population

Sample Characteristics	Patients With ASD, <i>n</i> = 274	Control Subjects, <i>n</i> = 219	Statistics	<i>p</i> Value
Site of Inclusion (C, K, M, N, R, U)	C: 45, K: 78, M: 21, N: 77, R: 20, U: 33	C: 32, K: 55, M: 18, N: 57, R: 18, U: 39	χ^2	NS
Age, Years, Mean (SD) [Range]	17 (5) [7–30]	17 (5) [6–30]	<i>t</i> test	NS
Sex Ratio (% of Males)	73%	64%	χ^2	<.05
Full Scale IQ, Mean (SD) [Range]	103 (19) [70–148]	108 (18) [70–142]	<i>t</i> test	<.05
Verbal IQ, Mean (SD) [Range]	102 (20) [70–160]	104 (18) [70–158]	<i>t</i> test	<.05
Performance IQ, Mean (SD) [Range]	104 (19) [70–150]	105 (20) [70–147]	<i>t</i> test	<.05
ADOS-2, Mean (SD) [Range]	Social affect: 6 (2) [1–10] Communication: 4 (2) [1–10] CSS total: 4 (3) [1–10]	NA	NA	NA
ADI-R, Mean (SD) [Range]	Social affect: 16 (6) [1–28] Communication: 12 (5) [0–25] RRB: 4 (2) [0–12]	NA	NA	NA
SRS-2 Score, Mean (SD) [Range] ^a	70 (12) [43–90]	NA	NA	NA
Diagnosis of ADHD, Yes/No, <i>n</i> ^b	98/144	NA	NA	NA
RBS-R Score, Mean (SD) [Range] ^c	15 (13) [0–73]	NA	NA	NA
SSP Score, Mean (SD) [Range] ^d	130 (36) [4–189]	NA	NA	NA

ADI-R, Autism Diagnostic Interview–Revised; ADOS-2, Autism Diagnostic Observation Schedule, Second Edition; ASD, autism spectrum disorder; C, Cambridge; CSS, calibrated severity score; K, King's College London; M, Mannheim; N, Nijmegen; NA, not applicable; NS, nonsignificant; R, Rome; RBS-R, Restricted Behavior Scale–Revised; RRB, restrictive and repetitive behaviors; SRS-2, Social Responsiveness Scale, Second Edition; SSP, Short Sensory Profile; U, Utrecht.

^aData missing for 50 individuals.

^bData missing for 32 individuals.

^cData missing for 56 individuals.

^dData missing for 59 individuals.

cerebellar parcellation or VBM cerebellar maps as predictive features. Balanced accuracy did not exceed 52% (Table S8).

Meta-analytic Approach and Site by Site Analysis

We compared cerebellar anatomy in individuals with autism and control subjects in each site of inclusion (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, 2 regions of interest were different in 2 sites: compared with control subjects, there was an increase in the volume of lobule VI in individuals with autism recruited in Rome ($p = .035$, uncorrected) and an increase in the volume of crus II ($p = .048$, 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 with control subjects in Nijmegen ($p = .041$, uncorrected).

It is important to note that the significant results obtained in 2 sites with the CERES pipeline were not significant using the SUIT pipeline (despite a good correlation between both measures reported in Figure S6). For all significant results, the confidence interval (Figure 4) was large compared with the full sample analysis and close to 0. The meta-analysis metrics (q statistic and I^2 ; Table 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 multi-centric cohort to reconcile previous results from the literature. We combined complementary statistical (both traditional group case-control paradigms and individual deviations using

Table 2. No Association of Autism With Cerebellar Parcellation—CERES Analysis

ROI	<i>p</i> Value	<i>t</i> Value	CI: Inf; Sup	Cohen's f^2					
				Group	Sex	Age	Site	ICV	FSIQ
Cerebellum	.3824	−0.87	−2.26; 0.87	0.002	0.022	0.040	0.117	0.293	0.005
Ant. Lobe	.8042	0.25	−0.24; 0.31	0.000	0.024	0.015	0.179	0.184	0.005
Lobule VI	.3276	−0.98	−0.59; 0.20	0.002	0.010	0.024	0.046	0.137	0.000
Crus I	.3076	−1.02	−0.91; 0.29	0.002	0.007	0.005	0.046	0.118	0.004
Crus II	.3616	−0.91	−0.59; 0.21	0.002	0.002	0.034	0.107	0.074	0.004
Lobule VIIb	.3627	−0.91	−0.32; 0.12	0.002	0.006	0.017	0.062	0.109	0.011
Post. Inf. Lobe	.8685	0.17	−0.51; 0.61	0.000	0.016	0.015	0.151	0.215	0.000

Group, sex, age, site of inclusion, ICV, and FSIQ were included as covariates.

Ant. Lobe, anterior lobe; FSIQ, Full Scale IQ; ICV, intracranial volume; Inf, inferior; Post. Inf. Lobe, posteroinferior lobe; ROI, region of interest; Sup, superior.

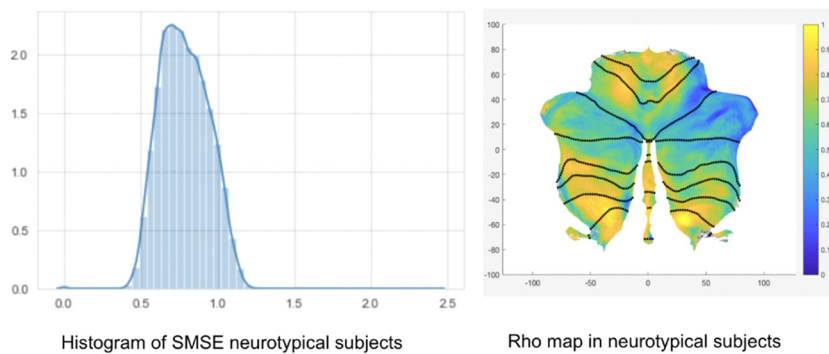


Figure 2. Goodness-of-fit of the normative model in control subjects. Symmetrical mean square error (SMSE) evaluates the goodness-of-fit of the model. Rho map shows the correlation between the predicted and the actual value for each voxel of the cerebellum. The relationship between the covariates (age, sex, intracranial volume, Full Scale IQ, and site of inclusion) and cerebellar anatomy is very strong, with nearly always positive correlation and SMSE < 1.

normative modeling and supervised learning) and neuroimaging (parcellation, VBM adapted to the cerebellum) methods to fully understand cerebellar anatomy in autism.

We found that regardless of the analytic technique we used, there was no case-control difference in 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.

Similar to 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 (35,36). However, to date, reports from MRI studies on cerebellar anatomy in autism are based on traditional case-control analysis and mostly are from relatively small samples. These studies typically reported cerebellar alterations in the crus I region (5,30), the anterior lobe (10), or the vermis (22,23,37), which were correlated to clinical dimensions of autism. A meta-analysis of 30 studies on cerebellar anatomy in autism (9) reported a weak but significant association between autism diagnosis and increased global (overall) cerebellar volume ($p = .049$, 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 with neurotypical control subjects. These results were consistent across 2 different parcellations methods and a voxelwise analysis. Parcellations were visually inspected by an expert rater blinded to 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 used (10,38,39). Cerebellar parcellation can be performed manually, semiautomatically, and fully automatically. Because of the heterogeneity of autism, it is critical to investigate its neuroanatomy in large multicenter samples to avoid false positive

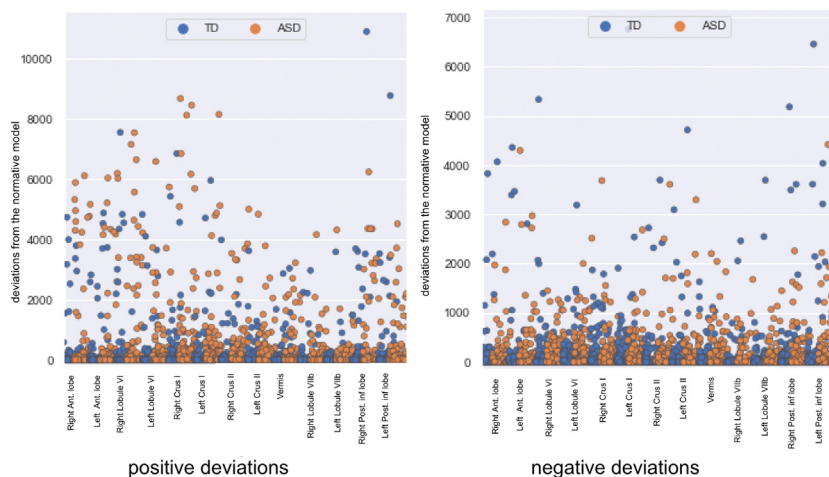


Figure 3. No difference of positive/negative deviations in the regions of interest of the cerebellum in autism versus neurotypical populations. Ant. Lobe, anterior lobe; ASD, autism spectrum disorder; Post. Inf. Lobe, posteroinferior lobe; TD, typically developing.

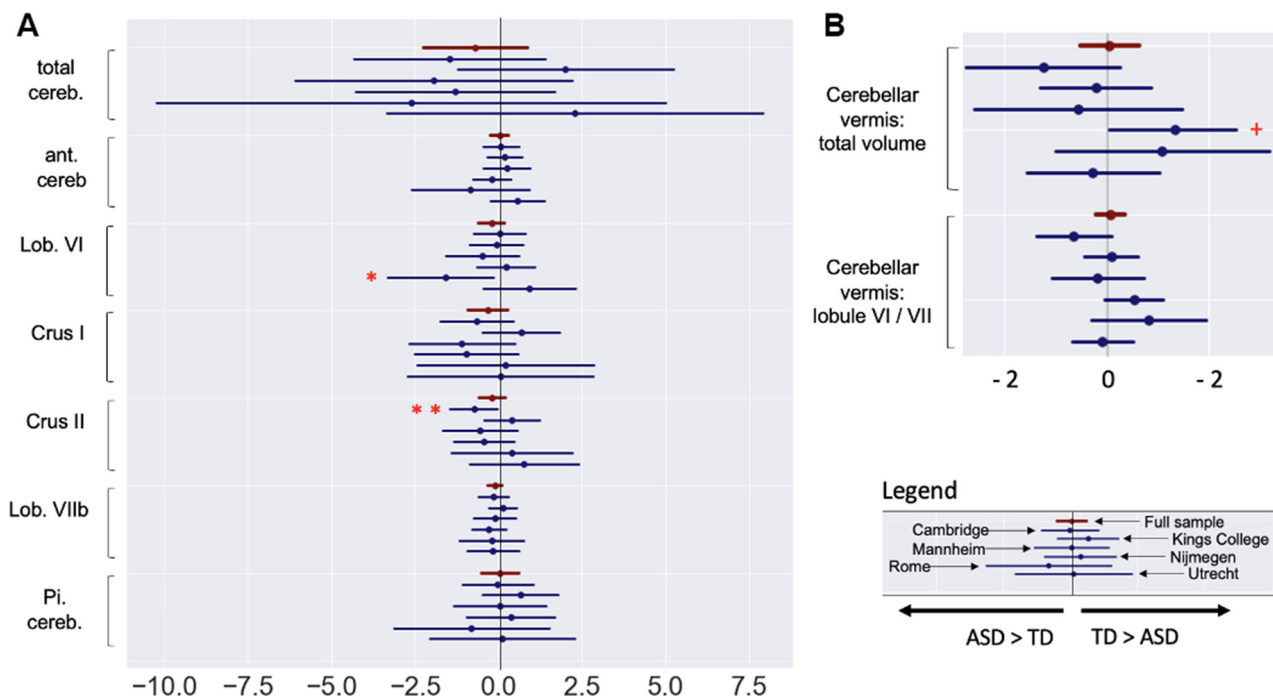


Figure 4. Cerebellar parcellation in each site of inclusion in individuals with autism spectrum disorder (ASD) and typically developing (TD) control subjects. **(A)** Effect of diagnosis (95% confidence intervals) for the total gray matter volume of the cerebellum and each subregion of the cerebellum (CERES pipeline) in the whole sample (dark red) and each site of inclusion (dark blue). **(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). The x-axis shows 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 for the whole sample (dark red). The y-axis shows the regions of the cerebellum. *uncorrected p value = .035; **uncorrected p value = .048; +uncorrected p value = .041. ant. cereb., anterior lobe of the cerebellum; cereb., cerebellum; Lob., lobule; Pi. cereb., posteroinferior cerebellum.

results (40). In addition, manual and semiautomated 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 (20,21). 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 (21). 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.* (20), where the vermis is merged into the cerebellar hemisphere. Thus, the crus II region, where we found only a moderate correlation between both methods, encompasses part of the vermis in the CERES pipeline, as compared with the SUIT pipeline where the vermis is isolated from the hemisphere. This difference of definition in cerebellar parcellation might partly explain the discrepant findings from previous studies. In our study, we analyzed 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 control subjects. In addition, we used the SUIT pipeline (21) 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 neocortex, in the posterior fossa) and its specific anatomical structure (high degree of folding), analysis of the cerebellum requires specific tools and parcellation algorithms. In this paper, an expert rater, blinded 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 control subjects. 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 (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 a 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, and 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 than the EU-AIMS sample, the severity of autistic symptoms had no influence on cerebellar structure. Finally, 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-analytic 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 (27). While our article 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. Finally, 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 control subjects. 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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