Evidence for normal extra-axial cerebrospinal fluid volume in autistic males from middle childhood to adulthood

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Abstract

Autism spectrum disorder has long been associated with a variety of organizational and developmental abnormalities in the brain. An increase in extra-axial cerebrospinal fluid volume in autistic individuals between the ages of 6 months and 4 years has been reported in recent studies.
Increased extra-axial cerebrospinal fluid volume was predictive of the diagnosis and severity of the autistic symptoms in all of them, irrespective of genetic risk for developing the disorder. In the present study, we explored the trajectory of extra-axial cerebrospinal fluid volume from childhood to adulthood in both autism and typical development. We hypothesized that an elevated extra-axial cerebrospinal fluid volume would be found in autism persisting throughout the age range studied. We tested the hypothesis by employing an accelerated, multi-cohort longitudinal data set of 189 individuals (97 autistic, 92 typically developing). Each individual had been scanned between 1 and 5 times, with scanning sessions separated by 2–3 years, for a total of 439 T1-weighted MRI scans. A linear mixed-effects model was used to compare developmental, age-related changes in extra-axial cerebrospinal fluid volume between groups. Inconsistent with our hypothesis, we found no group differences in extra-axial cerebrospinal fluid volume in this cohort of individuals 3 to 42 years of age. Our results suggest that extra-axial cerebrospinal fluid volume in autistic individuals is not increased compared with controls beyond four years of age.

Keywords
Autism spectrum disorder; Brain development; Cerebrospinal fluid; Extra-axial cerebrospinal fluid; MRI

1. Introduction

First identified by psychiatrist Leo Kanner in 1943, children who “had a need for sameness” and “resistance to change” were said to be “autistic” (Kanner, 1943). Autism spectrum disorder (ASD) refers to a clinically heterogeneous mix of individuals with a wide range of verbal ability (American Psychiatric Association, 2013). Despite the variability in verbal ability, ASD is characterized by well-documented core symptoms, which include impairments in social communication and the presence of restricted, repetitive behaviors and interests (American Psychiatric Association, 2013).

In addition to the aforementioned behavioral phenotypes, a variety of organizational and developmental abnormalities within the brain have been identified in ASD. These range from network inefficiencies in brain regions involved in low-level sensory processing (Lewis et al., 2017) to increased brain volume (Aylward et al., 2002; Courchesne et al., 2001; Hardan et al., 2001; Piven et al., 1996) and head size (Hazlett et al., 2005; Lainhart et al., 2006; Nordahl et al., 2011; Pardo et al., 2005). Remarkably, the latter finding was even observed by Leo Kanner in 1943 when he noted that five of his 11 cases, “had relatively large heads” (p. 248). Additional findings include abnormal lateralization of the brain with a lack of left lateralization in the structure and function of regions involved with language (Jouravlev et al., 2020; Lindell and Hudry, 2013; Nielsen et al., 2014), and hypoconnectivity within the default mode network (Di Martino et al., 2014). Furthermore, it has been found that the amygdala initially enlarges but fails to undergo the age-related increase found in typically developing (TD) children, with a larger amygdala predicting more severe symptomatology (Schumann et al., 2004).

Another feature of the brain that has shown promise as a potential biomarker of ASD is cerebrospinal fluid (CSF) volume (Shen, 2018). In addition to its significant role as
a hydromechanical protector of the cerebrum and adjacent structures, CSF is essential in maintaining homeostasis within the cerebral interstitial fluid (Sakka et al., 2011), including the glymphatic system (Iliff et al., 2012; Xie et al., 2013). As such, it regulates the electrolyte balance and eliminates toxic catabolites (Sakka et al., 2011). Moreover, in early development, CSF is responsible for cerebral development, distributing growth factors which signal progenitor cells to proliferate into immature neurons which later migrate to various areas of the cerebral cortex (Lehtinen et al., 2013).

Extra-axial CSF volume, or the amount of CSF surrounding the cerebral cortex in the subarachnoid space, is increased in autistic infants and children (or infants and children who later go on to be diagnosed with autism) between the ages of 6 months and 4 years. The relationship between extra-axial CSF and autism has been documented in at least three separate samples and studies: (1) infants between the ages of 6 and 24 months (Shen et al., 2013), (2) a larger independent sample of infants between the ages of 6 and 24 months (Shen et al., 2017), and (3) children between the ages of 2 and 4 years (Shen et al., 2018). The reliability of these results hinged not on genetic risk for developing autism, but whether the participants were later diagnosed with autism (Shen et al., 2018). Thus, the greater extra-axial CSF volume was not merely correlated with later diagnosis, but predictive of the diagnosis and severity of the autistic symptoms (Shen, 2018).

The elevated extra-axial CSF volume in autism that is present in early childhood raises the question as to whether the difference persists throughout later childhood and into adulthood. We hypothesized that an elevated extra-axial CSF volume would be found in autism compared with typical development, with that difference persisting throughout the lifespan. We analyzed data from an accelerated, multi-cohort longitudinal design to test our hypothesis. The accelerated, multi-cohort longitudinal design is suited to our research question in that it allows us to investigate developmental change over time across a variety of ages (Galbraith et al., 2017). However, this wider range of ages poses a problem for the implementation of the method used. The pipeline previously in use by Shen and colleagues (Shen et al., 2017) has been applied to this older sample. Nonetheless, as described hereafter, several precautionary steps have been taken to ensure that accuracy and reliability in this older sample are maintained.

2. Materials and methods

2.1. Participants

We analyzed a data set that includes participants previously reported in Alexander et al. (2007), Prigge et al. (2013), Prigge et al. (2018), and Zielinski et al. (2014). Further information on participant recruitment and diagnosis can be found elsewhere (Alexander et al., 2007; Prigge et al., 2013, 2018; Zielinski et al., 2014). The data reported on herein can be accessed on the National Database for Autism Research (NDAR). Additionally, all code employed in this study can be found on Github (https://github.com/peter3200/Autism_CSF_Scripts).

Participants initially included 112 male individuals with ASD, 4 female individuals with ASD, 119 TD male individuals, and 5 TD female individuals. After a quality-control process
(described below) and exclusion criteria of age greater than 43 and females, the final sample included in the analysis consisted of 97 autistic male individuals and 92 TD male individuals. The exclusion criterion of age greater than 43 was selected due to the lack of matched controls for participants older than 43. Additional demographic information can be found in Table 1. In summary, ASD mean age was 19.42 years, range 3–42.17 years; control mean age was 20.80 years, range 3.42–41.83 years; overall mean age was 19.99 years (see Fig. 1). As previously reported, follow-up data are unavailable for participants with a single time point for a variety of reasons including technical (such as orthodontic braces), educational (such as out-of-state college), and social (such as long-term family or community obligations; Zielinski et al., 2014). Furthermore, a large number of new controls were recruited at time point five, so some participants only have one scan available because they were recruited later in the study. For participants with multiple scans, the average interscan interval was 3.18 years (range 2–5.33 years).

Autistic participants and typically developing participants did not significantly differ in mean age ($t(408.58) = -1.61, p = .11$) or mean interscan interval ($t(231.39) = 1.75, p = .08$). There was a significant difference between groups on available intelligence quotient (IQ) measures ($p < .001$). Details regarding IQ measures have been previously reported (Prigge et al., 2013; Zielinski et al., 2014). Using the criterion of an average full-scale IQ score less than or equal to 79 to operationalize low verbal and cognitive performance (Gabrielsen et al., 2018), it was found that 31 scans came from 10 autistic participants with low verbal and cognitive performance and two scans came from one typically developing control with low verbal and cognitive performance. Additionally, 214 scans came from 79 autistic participants with high verbal and cognitive performance and 141 scans came from 53 control participants with high verbal and cognitive performance.

Additionally, Autism Diagnostic Observation Schedule (ADOS) calibrated severity scores (CSS) at entry are reported in Table 1. The ADOS is the gold standard in assessing autism, and higher scores denote more severe symptoms. The ADOSes were administered by trained clinicians or research reliable senior study staff and the ADOS CSS scores were calculated based on ADOS module and participant age (Gotham et al., 2009). In order to provide a more complete description of the autistic participants and their ADOS CSS scores, ADOS CSS scores taken at time 5 have been introduced when a participant did not have an ADOS CSS at entry score. Additionally, some participants were clinical patients of one of the senior study staff members and the ADOS was not administered to these participants since their ASD diagnosis had been confirmed prior to their enrollment in the study. Autism Diagnostic Interview-Revised (ADI-R) scores are also reported, and these scores are the participants’ most abnormal age 4–5 scores and act as a summary of autism severity during childhood. Details regarding the ADOS and ADI-R assessments as criteria for the autism diagnosis can be found in prior studies (Prigge et al., 2013; Zielinski et al., 2014).

### 2.2. Image acquisition procedure

Scan acquisition took place on a Siemens Trio 3T scanner. At time point one, an 8-channel, receive-only RF head coil was used to acquire sagittal 3D MPRAGE T1-weighted images (inversion time = 1100 ms, echo time = 2.93 ms, repetition time = 1800 ms, flip angle = 12°,
field of view = 256 mm, slice thickness = 1.0 mm, 160 slices). At all subsequent time points, a 12-channel, receive-only RF head coil was used to acquire 3D MPRAGE T1-weighted images (inversion time = 900 ms, echo time = 2.91 ms, repetition time = 2300 ms, flip angle = 9°, field of view = 256 mm, slice thickness = 1.2 mm, 160 slices). This head coil upgrade in addition to changes in magnetic resonance sequence parameters following the first time point warrant a ‘head coil’ covariate, which was included in our analyses.

### 2.3. Image processing

This data set is composed of 439 T1-weighted structural MRI scans collected at the University of Utah, following an accelerated, multi-cohort longitudinal design. One scan was available on 28 ASD and 48 typically developing participants, two scans on 20 ASD and 14 typically developing participants, three on 17 ASD and 16 typically developing participants, four on 23 ASD and 11 typically developing participants, five on 9 ASD and 3 typically developing participants. The clinical severity of the autistic participant did not preclude their ability to participate in multiple scans, as there was no difference in autism severity between those who had only one scan versus those with multiple scans ($t(91) = 0.45, p = .66$).

Extra-axial CSF was defined as CSF within the subarachnoid space surrounding the dorsal convexity (thus excluding all spinal and ventricular CSF), with a ventral boundary at the plane of the anterior and posterior commissures (Shen et al., 2013, 2017, 2018). In order to derive the volume of extra-axial CSF from these scans, we implemented a neuroimaging pipeline that included the following steps. To begin, the data underwent preprocessing via the ANTs tool N4BiasFieldCorrection (Sled et al., 1998). This step is necessary to correct for bias field signal, which can result in non-uniformities in image intensity. After this step, resampling via the package c3d (Yushkevich et al., 2006) was undertaken since images acquired following the first time point had a slice thickness of 1.2 mm. Voxel dimensions for images collected at time point one were 1.0 mm by 0.5 mm by 0.5 mm while the dimensions for time points 2–5 were 1.2 mm by 0.5 mm by 0.5 mm. These were resampled to 1.0 mm isotropic voxels.

Following these preprocessing steps, we processed the structural images with the Automatic Extra-axial Cerebrospinal Fluid (Auto EACSF) pipeline version 1.7.7 (Shen et al., 2017). The pipeline registers, skull-strips, and then segments each T1-weighted image. After segmentation, with a ventricle mask and template as input, the tool generates a progressive series of images such that any ventricular or cistern CSF is gradually eradicated from one image to the next. After inspecting the intermediate output files, it was determined that the output file with the suffix “MID02” was most accurate at approximating extra-axial CSF (later output files such as the “QCistern” file tended to be overaggressive in stripping away extra-axial CSF). Finally, the Computational Morphometry Toolkit (CMTK) was employed to calculate the number of CSF voxels in each MID02 file. See Github ([https://github.com/peter3200/Autism_CSF_Scripts](https://github.com/peter3200/Autism_CSF_Scripts)) for all code employed to run preprocessing and pipeline applications.

Total brain volume was also extracted as a control measure since a significant difference was found between the ASD and TD groups in previous work by Shen and colleagues (Shen et al., 2013, 2017, 2018). To extract the total brain volume, images were automatically
processed with the cross-sectional stream in FreeSurfer 6.0.0, which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/).

2.4. Reliability of the processing pipeline

Since the Auto EACSF pipeline has never before been used on individuals older than four years, the test-retest reliability of Auto EACSF was examined through the use of 28 Human Connectome Project subjects (Van Essen et al., 2012). These subjects were selected since they each had two T1-weighted scans. These scans were used to assess the reliability of the pipelines with the same parameters used for the later processing of the present study’s longitudinal data. The pipeline was found to be highly reliable (see Fig. 2). For the extra-axial CSF volume, the intra-class correlation (ICC) was 0.96 ($F(27, 27) = 47, p < .001, 95\% CI [0.92, 0.98]$).

2.5. Visual quality-control

Both the raw T1-weighted images and Auto EACSF output images for all participants underwent visual quality-control by a single rater (MP) who was blinded to the identities of the images. Raw T1-weighted images were assessed for quality and the presence of motion artifact, using a four-scale rating system similar to those previously reported (Backhausen et al., 2016; Murphy et al., 2020). The output images from Auto EACSF were also assessed for the quality of segmentation using the scale and standard images developed by Murphy et al. (2020). Only subjects with a score of zero (no abnormalities in segmentation) or one (minor under- or overestimation of extra-axial CSF in one region) were included in this study (Murphy et al., 2020). Excluding females and participants 43 and older due to insufficient participants and scans, we started with 635 scans (ASD: 378, TD: 257). Of these, 193 scans failed the visual quality-control protocol (ASD: 120, TD: 73). After the visual quality-control, there was a group difference in the extra-axial CSF segmentation quality-control rating (as assessed with a mixed effects model: $t(187) = 2.30, p = .02$). This difference was such that scans from autistic individuals were of worse quality compared with scans from typically developing individuals, so a quality-control covariate was included in the model.

2.6. Statistical analysis

Mixed-effects models were used to examine longitudinal age-related changes in extra-axial CSF volume for the ASD and TD groups. This type of model provides added flexibility in modeling both sources of variance and correlation within the data, and is particularly suited to data with repeated measures. Longitudinal extra-axial CSF volume was modeled with participants contributing the random effects, age at scan as a fixed effect and the following covariates: age, age$^2$, age$^3$, diagnostic group, diagnostic group by age, diagnostic group by age$^2$, diagnostic group by age$^3$, extra-axial CSF segmentation quality-control rating, total brain volume, total brain volume$^2$, and head coil. The age, age$^2$, age$^3$, total brain volume, and total brain volume$^2$ covariates were mean-centered. Covariates were selected using a theoretical approach. For example, the age, age$^2$, and age$^3$ covariates were selected due to the sigmoidal-shaped curve generated by a loess smoother when extra-axial CSF volume by age was plotted (see Fig. 3). Analyses were performed in R 4.0.2 (R Core Team, 2020) using the nlme package (Pinheiro et al., 2020). An analysis examining the relationship between
extra-axial CSF volume and total mean cortical thickness, which was operationalized as the combination of the mean cortical thicknesses from each hemisphere divided by two. Sub-analyses using nearest neighbor matching between the autism and control scans on the basis of age or full-scale IQ were implemented using the R package MatchIt (Ho et al., 2011).

3. Results

3.1. Group and age effects on extra-axial CSF volume

Starting at age three, we estimate an average extra-axial CSF volume of approximately 45 cm$^3$ for both the autistic and TD groups. Between 3 and 20 years, the volume of extra-axial CSF approximately doubles. After age 20, the extra-axial CSF volumes level off through age 42 (see Fig. 3). A significant effect of age on extra-axial CSF volume was found ($t(240) = 14.57$, $p < .001$) in addition to a significant age$^3$ effect ($t(240) = −2.14$, $p = .03$). The trajectories of extra-axial CSF volume between 3 and 42 years are similar for the autistic and TD groups (group x age interaction, $t(240) = −0.49$, $p = .62$). Also, no significant differences between the autistic and control groups are observed after recentering age at 10 ($t(187) = −1.00$, $p = .32$), 20 ($t(187) = −1.19$, $p = .23$), and 30 ($t(187) = −1.63$, $p = .11$) years.

3.2. Additional analyses investigating other predictors of extra-axial CSF

In addition to the primary analyses described above, we also investigated other possible factors associated with extra-axial CSF volume. Given the anatomical proximity of extra-axial CSF to the cortex, we chose to examine the relationship between mean cortical thickness and extra-axial CSF volume using a multilevel model incorporating the following predictors: total mean cortical thickness, head coil, extra-axial CSF segmentation quality-control rating, diagnostic group, total brain volume, and total brain volume$^2$. Both total mean cortical thickness and extra-axial CSF volume were adjusted by regressing out the effects of head coil, extra-axial CSF segmentation quality-control rating, total brain volume, and total brain volume$^2$. A significant negative relationship between total mean cortical thickness and extra-axial CSF volume was found ($t(245) = −9.48$, $p < .001$), such that as the total mean cortical thickness estimate decreases, extra-axial CSF volume increases. This is demonstrated well in adolescence, during which we see a large increase in the volume of extra-axial CSF (see Fig. 3) and a simultaneous decrease in estimated total mean cortical thickness (see Fig. 4A and B). This finding is likely caused by the pruning of the cortical mantle, which is known to thin across adolescence (Gogtay et al., 2004). As the volume of gray matter decreases due to this pruning event, the space between the pial surface and dura mater would presumably increase, creating a deficit that could then be filled with an increased volume of extra-axial CSF.

In order to understand if age differences between the autism and typically developing groups contributed to the null finding, a multilevel model using 366 age-matched scans (177 participants) was implemented. We found results similar to the unmatched model reported previously, with a statistically significant age covariate ($t(179) = 13.35$, $p < .001$) and no significant group ($t(175) = −1.39$, $p = .17$) or group by age interaction ($t(179) = −0.34$, $p = .73$).
Likewise, given the significant difference in mean full-scale IQ scores between the autism and control groups, a separate sub-analysis of 218 full-scale IQ-matched scans (106 participants) was used. Similar conclusions were reached once more, with statistically significant age ($t(102) = 10.21, p < .001$), and age$^2$ effects ($t(102) = -4.38, p < .001$) but a nonsignificant group effect ($t(104) = -1.26, p = .21$) and age by group interaction ($t(102) = 1.69, p = .09$).

To address the potential relationship between IQ and extra-axial CSF volume, a multilevel model incorporating the full-scale IQ score from each time point as a predictor was used ($N = 343$ scans from 143 participants). Full-scale IQ was not a significant predictor of extra-axial CSF volume ($t(189) = -0.57, p = .57$).

Similarly, to address the potential relationship between ASD severity and extra-axial CSF volume, a multilevel model incorporating ADOS CSS entry scores as a predictor was used ($N = 248$ scans from 93 autistic individuals). The ADOS CSS entry predictor was not statistically significant ($t(91) = -0.23, p = .82$).

Additionally, to investigate head coil as a potential confound, a mixed effects model using only the subjects with the sequence/head coil from times 2–5 ($N = 176$ participants, 374 scans) arrived at the same general results as described above. There were significant fixed effects for the intercept ($t(189) = 31.89, p < .001$), age ($t(189) = 13.89, p < .001$), and age$^2$ ($t(189) = -4.66, p < .001$). There were not statistically significant fixed effects for diagnostic group ($t(174) = -1.46, p = .15$) or the diagnostic group by age interaction ($t(189) = 0.04, p = .97$).

4. Discussion

In this study, we examined the developmental trajectory of extra-axial CSF from early childhood through adulthood in typically developing and autistic individuals. Our results indicate that both groups experience a nonlinear increase in extra-axial CSF volume in this cohort of individuals 3 to 42 years of age. Contrary to our hypothesis, we find no difference in the trajectory of extra-axial CSF volume in individuals between ages 3 and 42 years. These results extend the extra-axial CSF volume research of Shen and colleagues in an accelerated, multi-cohort longitudinal design. Previously, extra-axial CSF volume in relation to autism has been investigated in samples of children 6–24 months (Shen et al., 2013, 2017) and 2–4 years (Shen et al., 2018). Collectively, these studies have indicated that increased extra-axial CSF volume may act as a biomarker or stratification marker for autism. To further this idea and increase the specificity of this potential biomarker to autism, additional longitudinal work examining extra-axial CSF volume and risk for schizophrenia was undertaken (Murphy et al., 2020; Xu and Lehtinen, 2020). No significant relationship between schizophrenia risk and extra-axial CSF volume at ages one and two years was found (Murphy et al., 2020; Xu and Lehtinen, 2020). The present study extends the previously examined timeframe and suggests that extra-axial CSF volume as a potential biomarker for autism may be constrained to a specific developmental period before normalizing later in childhood.
4.1. Mechanisms of extra-axial CSF volume in autism

The first three years in human development are incredibly important to the emergence and identification of autistic symptoms (Estes et al., 2015; Landa et al., 2013; Zwaigenbaum et al., 2013). Beyond behavioral markers, many neuroanatomical findings arise during this period, including aberrant white matter integrity in the genu of the corpus callosum and cerebellar peduncles (Shen and Piven, 2017) and enlarged head circumference and brain volume (Aylward et al., 2002; Courchesne et al., 2001; Hardan et al., 2001; Hazlett et al., 2005, 2017; Nordahl et al., 2011; Pardo et al., 2005; Piven et al., 1996; Shen et al., 2013, 2018). This consistent finding of increased brain volume is associated with an increase in cortical surface area, but not cortical thickness (Hazlett et al., 2017).

Recently, it has been proposed that this increase in head size and brain volume in autism may be related to a decrease in the diffusion or absorption of CSF, particularly extra-axial CSF. This could occur via impairment in cerebro-venous outflow (Sainz et al., 2019), the underdevelopment of the arachnoid granulations due to immaturity (Shen and Piven, 2017; Xu and Lehtinen, 2020), or impediment to the meningeal lymphatics system (Kapoor et al., 2008). If this were to occur at a critical developmental juncture (such as between years one and two), cortical development could be impacted in negative ways. Some of the earliest roles of CSF include providing a supportive environment for the brain and distributing factors implicated in neurogenesis, progenitor survival, and progenitor proliferation (Fame and Lehtinen, 2020). Thus, the accumulation and stagnation of CSF could result in the accrual of metabolic byproducts in the brain (Shen and Piven, 2017). Furthermore, this would have a direct impact on cortical development, altering neurogenesis and prompting the premature migration of progenitor cells (Shen and Piven, 2017).

As an additional potential mechanism influencing the volume of extra-axial CSF, cortical thickness is a viable candidate. In an exploratory analysis, we found a negative relationship between mean cortical thickness and the volume of extra-axial CSF in autistic and typically developing participants 3 to 42 years of age. This finding corroborates that of a decrease in mean cortical thickness across adolescence (Gogtay et al., 2004; Raznahan et al., 2011; Walhovd et al., 2016) while simultaneously supporting the role of mean cortical thickness as a potential causal mechanism for the dramatic increase in extra-axial CSF volume during this developmental period. This would operate such that as the cortical mantle decreases in volume, a deficit in the subarachnoid space would presumably result which could then be filled with an increase in the volume of extra-axial CSF. However, as this is an exploratory analysis in a single sample, additional investigation is needed to substantiate these results. Furthermore, it should be noted that the mechanisms driving the trajectories of extra-axial CSF volume in infancy may be distinct from those in later childhood, adolescence, and adulthood. To this end, additional research into the relationship between cortical thickness and extra-axial CSF volume in infancy would be beneficial.

4.2. Normalization of extra-axial CSF volume in autism

While the direct causal mechanisms resulting in the normalization of extra-axial CSF volume remain unknown, processes related to maturation appear to be at play. In conjunction with previous work, our results indicate after age four is the approximate timeframe...
in which the normalization of extra-axial CSF volume in autistic individuals occurs. Interestingly, this corresponds with findings regarding the normalization of previously accelerated head and brain growth in autism. In a review on this subject, Ecker et al. (2015) noted that the increased brain volume in autism typically resolves around ages six to eight. Similarly, in a longitudinal study, Aylward et al. (2002) found that enlarged brain volume and head circumference in autism normalizes by approximately age 12.

Beyond childhood and adolescence, the brain continues to undergo dynamic structural and functional changes (reviewed in Somerville, 2016). One study of note found that volumetric changes for a variety of structures, including the lateral ventricles and white matter, did not plateau between ages 15 and 90 (Walhovd et al., 2005). Similarly, we observed changes in the volume of extra-axial CSF in emerging adulthood and beyond. And, if the negative relationship between extra-axial CSF volume and cortical thickness in adolescence is any indicator, ongoing structural changes in the brain are likely relevant to the positive trajectory of extra-axial CSF volume observed in adulthood.

4.3. Limitations and future directions

Extra-axial CSF in this study was operationalized in accordance with previous work by Shen and colleagues in order to best extend their work. Unfortunately, the use of a ventral boundary at the anterior commissure-posterior commissure line leaves out a large quantity of extra-axial CSF, including CSF surrounding the temporal and occipital lobes. However, we note that a pipeline using a localized, cortical surface analysis is currently in development and described by Mostapha and colleagues, although the pipeline has not yet been made publicly available (Mostapha et al., 2020). This new pipeline may resolve these concerns and allow for future investigation into total extra-axial CSF volume and its relationship to autism.

This analysis utilized a pipeline never before used on MRI scans from adolescents or adults. This comes with a host of issues, including the fact that we do not know that the anterior commissure-posterior commissure plane retains the exact same position in the brain and distance from the top of the intracranial cavity as the brain remodels from childhood into young adulthood. To address this and similar issues, care was taken to understand the test-retest reliability of the pipeline on scans from adults, and we visually quality-controlled the output from each image. Despite these steps, the use of this pipeline on this sample may have introduced additional variance into the data.

Additionally, it should be noted that the sample included in the analysis consisted entirely of male participants, which limits the generalizability of these results. Going forward, studies should investigate the developmental trajectory of extra-axial CSF in females in addition to potential sex differences in the trajectory of extra-axial CSF across the lifespan in both autistic and typically developing individuals. Furthermore, while a sub-analysis using scans matched for full-scale IQ provided similar results, we cannot eliminate the discrepancy in IQ between the ASD and TD groups as a potential influence on our results. Along these lines, it should also be noted that the overwhelming majority of scans came from participants with high verbal and cognitive performance, which may account for our null finding.
Finally, while examining individual variability is an important next step in understanding a developmental disorder as heterogeneous as autism (Wolff et al., 2018), extreme individual deviations in the rate of change in extra-axial CSF volume were not examined here. Rather, the purpose of this study was to examine group trends over time. Nonetheless, future studies will need to address individual differences in extra-axial CSF trajectories in both typically developing and autistic individuals.

5. Conclusions

In contrast to our hypothesis and previous reports of increased extra-axial CSF volume in autistic individuals between six months and four years, we found no group differences in extra-axial CSF volume between 3 and 42 years. When considered in combination with previous reports, our results suggest that the increased extra-axial CSF volume in young autistic children normalizes after age 4.

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Fig. 1.
Participant age at scan. Following the quality-control procedures, participants included 97 autistic male individuals and 92 TD male individuals (ASD mean age was 19.42 years, range 3–42.17 years; TD mean age was 20.80 years, range 3.42–41.83 years; overall mean age was 19.99 years; overall mean age 19.99 years). One scan was available on 28 ASD and 48 typically developing participants, two scans on 20 ASD and 14 typically developing participants, three on 17 ASD and 16 typically developing participants, four on 23 ASD and 11 typically developing participants, five on 9 ASD and 3 typically developing participants. Each scan is represented by a circle and repeated scans for a participant are connected by a horizontal line.
Fig. 2.
Extra-axial CSF volume estimates are reliable. Depicted is the test-retest reliability of extra-axial CSF volume, which was derived using Auto EACSF version 1.7.7 and 28 Human Connectome Project subjects (Van Essen et al., 2013). Two T1-weighted scans from each subject were employed to assess the reliability of the pipeline with the same parameters used for the later processing of the longitudinal data examined in this study. Each circle represents a single subject and the dotted line is the identity line for reference. The intraclass correlation was high (ICC = 0.96, $F(27, 27) = 47$, $p < .001$, 95% CI [0.92, 0.98]).
Fig. 3.
No significant difference in extra-axial CSF volumes between the autistic and TD individuals. Depicted is the nonlinear trajectory of the extra-axial CSF volumes between 3 and 42 years. No significant difference between the autism and control groups was found (group x age interaction, $t(240) = -0.49$, $p = .62$). The extra-axial CSF volumes were adjusted by regressing out the effects of head coil, extra-axial CSF segmentation quality-control rating, total brain volume and total brain volume$^2$. A loess smoother was used to fit the developmental trajectories for both groups. Each data point represents a single scan, and multiple data points connected by a line represent repeated scans for a single participant.
Fig. 4.
The relationship between cortical thickness, age, and extra-axial CSF volume. Panel A depicts the individual trajectories of total mean cortical thickness between 3 and 42 years. The total mean cortical thickness estimates were adjusted by regressing out the effects of head coil, extra-axial CSF segmentation quality-control rating, total brain volume, and total brain volume\(^2\). A loess smoother was used to fit the trajectories. Each data point represents a single scan, and multiple data points connected by a line represent repeated scans for a single participant. Panel B depicts the negative relationship between total mean cortical thickness and extra-axial CSF volume between 3 and 42 years. Cortical thickness was a significant predictor of extra-axial CSF volume ($t(245) = -9.48$, $p < .001$). Both total mean cortical thickness and extra-axial CSF volume were adjusted by regressing out the effects of head coil, extra-axial CSF segmentation quality-control rating, total brain volume, and total brain volume\(^2\). A loess smoother was used to fit the trajectories. Each data point represents a single scan, and multiple data points connected by a line represent repeated scans for a single participant.
Table 1

Demographics.

|                                | Autism, N = 97 | TD, N = 92 | Group Comparison |
|--------------------------------|----------------|------------|------------------|
|                                | Mean (SD)      | Range      | Mean (SD)        | Range | t    | p    |
| Mean Age (years)               | 19.42 (9.23)   | 3–42.17    | 20.80 (8.57)     | 3.42–41.83 | −1.61 | 0.11 |
| Interscan interval (years)     | 3.22 (0.47)    | 2.08–5.33  | 3.11 (0.62)      | 2–5.33  | 1.75  | 0.08 |
| Mean Performance IQ<sup>a</sup> | 102.58 (17.01) | 59.5–138   | 113.68 (13.53)   | 77–140.50 | −4.22 | < 0.001 |
| Mean Verbal IQ<sup>b</sup>     | 97.96 (19.62)  | 56–138     | 113.16 (13.68)   | 74–146.33 | −5.32 | < 0.001 |
| Mean Full-scale IQ<sup>c</sup> | 100.58 (17.01) | 65–136.5   | 115.64 (13.52)   | 78–142.50 | −5.85 | < 0.001 |
| ADOS CSS at entry<sup>d</sup>  | 8.30 (1.54)    | 3–10       | –                | –       | –     | –    |
| ADI-R<sup>e</sup>              | 27.59 (7.43)   | 11–40      | –                | –       | –     | –    |

TD = Typically developing.

<sup>a</sup>Mean Performance IQ: Autism N = 87, typically developing controls N = 51.

<sup>b</sup>Mean Verbal IQ: Autism N = 86, typically developing controls N = 51.

<sup>c</sup>Mean Full-scale IQ: Autism N = 89, typically developing controls N = 54.

<sup>d</sup>ADOS CSS at entry: Autism N = 93. ADOS CSS scores at time 5 have been introduced when a participant did not have an ADOS CSS at entry score.

<sup>e</sup>ADI-R: Autism N = 73.