Increased Extra-axial Cerebrospinal Fluid in High-Risk Infants who Later Develop Autism

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Abstract

Background—We previously reported that infants who developed ASD had increased CSF in the subarachnoid space (i.e., extra-axial CSF) from 6–24 months of age (1). We attempt to confirm and extend this finding in a larger, independent sample.

Methods—A longitudinal MRI study of infants at-risk for ASD was carried out on 343 infants, who underwent neuroimaging at 6, 12, and 24 months; 221 were high-risk for ASD because of an older sibling with ASD; 122 were low-risk with no family history of ASD. Forty-seven infants were diagnosed with ASD at 24 months and were compared with 174 high-risk and 122 low-risk infants without ASD.

Results—Infants who developed ASD had significantly greater extra-axial CSF volume at 6 months compared to both comparison groups without ASD (18% greater than high-risk infants without ASD; Cohen's d=0.54). Extra-axial CSF volume remained elevated through 24 months (d=0.46). Infants with more severe autism symptoms had an even greater volume of extra-axial CSF from 6–24 months (24% greater at 6 months, d=0.70; 15% greater at 24 months, d=0.70). Extra-axial CSF volume at 6 months predicted which high-risk infants would be diagnosed with ASD at 24 months with an overall accuracy of 69% and corresponding 66% sensitivity and 68% specificity, which was fully cross-validated in a separate sample.

Conclusions—This study confirms and extends previous findings that increased extra-axial CSF is detectable at 6 months in high-risk infants who develop ASD. Future studies will address whether this anomaly is a contributing factor to the etiology of ASD or an early risk marker for ASD.

Keywords

INTRODUCTION

Autism spectrum disorder (ASD) is characterized by impairments in social communication and the presence of repetitive stereotyped behaviors beginning in early childhood and typically extending throughout life (2). ASD affects about 1–2% of children worldwide (3–5). Younger siblings of children with ASD are at substantially increased risk for developing ASD and offer an important strategy to discover early risk markers in a population unselected for having ASD (6). There are currently no biomarkers detectable in the first year of life that distinguish children who develop ASD from those who do not. Moreover, studies of high-risk infants have demonstrated that the defining behavioral features of ASD generally unfold in the latter part of the first and the second years of life (7–9).

Shen *et al.* (1) reported that high-risk infants who later developed ASD had increased extra-axial cerebrospinal fluid (EA-CSF) volume from 6–24 months, which was associated with autism severity at 36 months. Extra-axial fluid is defined as CSF in the subarachnoid space, surrounding the cortical convexities (10–12). While increased EA-CSF had been previously associated with impaired motor function (13–16), it had not been previously examined in relationship to ASD. Our initial report raised the possibility that dysregulation of CSF flow during the first year of life may play some role in the early pathogenesis of ASD, and/or provide a marker of an underlying process that contributes to ASD. The importance of CSF and its role in brain development has been highlighted in recent years (17). Once thought to merely provide a protective cushion for the brain, CSF has been found to play a critical role in the transport of growth factors that regulate progenitor cell production (18) and neuronal differentiation (19). In addition, as CSF circulates through the developing brain, it removes inflammatory cytokines and proteins secreted by neurons that can otherwise accumulate and have a pathological effect on brain development (20; 21).

In this study we sought to confirm and extend these findings in a larger, independent sample of infants at high- and low- familial risk for ASD (HR and LR infants, respectively), as part of the Infant Brain Imaging Study (IBIS) (22; 23). The current study has several important differences and advances over the original study: (1) an independent sample; (2) multi-site study drawn from four clinical sites across the United States; (3) a sample size roughly seven times larger than the original sample; (4) a different image acquisition protocol than the original study (harmonized across the four IBIS sites); and (5) a fully automated image analysis procedure to quantify EA-CSF volume. Based on findings from Shen et al. (1), we hypothesized that (1) HR infants later diagnosed with ASD (HR-ASD) would show increased EA-CSF volume at 6 months, compared to HR and LR infants who do not develop ASD (HR-negative and LR-negative, respectively); (2) HR-ASD infants would show persistently increased EA-CSF through 24 months; and (3) increased EA-CSF would be associated with autism severity as well as early motor deficits.

METHODS AND MATERIALS

Participants

Infants at high and low familial risk for ASD were enrolled at four clinical sites (University of North Carolina, University of Washington, Washington University, and Children's Hospital of Philadelphia) (23). HR infants had an older sibling with a clinical diagnosis of ASD, corroborated by the Autism Diagnostic Interview-Revised (ADI-R); (24). LR infants had a typically developing older sibling and no 1st or 2nd degree relatives with intellectual/psychiatric disorders (9). See Supplement for full inclusion/exclusion criteria. Parents provided informed consent, and the institutional review boards at each site approved the research protocol.

Assessment

Infants were assessed at 6, 12 and 24 months with an MRI and a behavioral battery that included measures of cognitive development (Mullen Scales of Early Learning) (25) and adaptive functioning (Vineland Adaptive Scales) (26). DSM-IV-TR criteria (27) and the

Autism Diagnostic Observation Schedule-G (ADOS) (28) were administered to all participants at 24 months. The ADI-R was administered at 24 months to all parents of high-risk infants and to all low-risk infants with clinical concerns. At 24 months, infants were classified as having ASD based on expert clinical judgment using DSM-IV-TR criteria (27) and all available clinical information, including the ADOS (28), ADI-R, and other behavioral measures. Further details on the assessment and diagnostic procedures can be found in Estes et al., 2015 (9). A small number of LR infants who met criteria for ASD (N=3) were excluded from the analysis because they were too few to constitute a comparison group and to keep the study design focused on ASD in the context of familial risk for ASD.

Infants were included in the analysis if the infant: (1) had a successful, high quality MRI at least at the initial 6 month visit; and (2) was assessed for an ASD diagnosis at the 24 month visit. A total of 343 infants (221 HR; 122 LR) met these criteria and were included in the analysis, yielding three outcome groups: [1] HR-ASD (N=47; 42 male, 5 female); [2] HR-negative (N=174; 95 male, 79 female); and [3] LR-negative (N=122; 76 male, 46 female). Table 1 provides a description of participant characteristics on the primary behavioral measures. Table 2 lists the number of MRI scans in the analysis at 6, 12, and 24 months.

By virtue of the large sample of infants at risk, we conducted follow-up analyses to assess whether subgroups of HR-ASD subjects, defined on the basis of autism symptom severity, differed in their volume of EA-CSF. The HR-ASD group (N=47) was stratified into subgroups according to established, empirically derived categories on the ADOS. Lord and colleagues established the subgrouping algorithm, which combines the scores on two ADOS domains (Social Affect and Restricted, Repetitive Behaviors) to derive the cutoff threshold that yields reliable autism subgroups (29). We applied this same ADOS threshold (29) to stratify the infants in the ASD group into those with ADOS scores above the threshold (ASD-High subgroup; N=23) and below (ASD-Moderate subgroup; N=24). This approach is consistent with previous publications on this sample (9).

MRI Acquisition

Imaging data were collected during natural sleep at 6, 12, and 24 months (Table 2). T1- and T2-weighted scans ($1mm^3$ voxels) were acquired. Description of the MRI acquisition, neuroradiological review, quality control, and cross-site reliability are detailed in a previous publication on this sample (22) and the Supplement.

Image Analysis and Quantification of Extra-axial CSF and Lateral Ventricles

In our earlier study, segmentation of EA-CSF was carried out manually (1). However, given the far greater number of scans in the current study, manual segmentation was not practical. Therefore, an automated algorithm to quantify EA-CSF and lateral ventricle (LV) volumes was developed based on the criteria used in the manual segmentation. (See Supplement for details on quantification and validation steps.) Ninety-nine percent of scans met quality inspection criteria for inclusion in the final analysis (N=804 scans; Table 2). The automated method showed a high correlation with the manual method (ICC=0.80). Figure 1

demonstrates an example of the resulting EA-CSF segmentation from the automated method.

Statistical Analysis

A longitudinal mixed effects model for repeated measures with unstructured covariance matrices was employed to analyze trajectories of EA-CSF and LV volume from 6 to 24 months of age. This analytic method is suitable for an unbalanced design and allows for missing values in a longitudinal study. Independent variables of interest included main effect of group, linear effect of age, quadratic effect of age (age^2), sex, and group interactions with each of these variables. Total cerebral volume (TCV) was included as a covariate given its relationship to EA-CSF and LV volumes (1) and to control for possible differences in brain size. Scan site was included as another control variable. Following significant omnibus results of the primary model described above, Bonferroni-corrected pairwise comparisons tested for cross-sectional group differences at each time point (6, 12 and 24 months), and estimated marginal means and the pooled standard deviation were generated to compute Cohen's *d* effect sizes. Percent differences in model-adjusted volumes at each time point and Cohen's *d* effect sizes are reported relative to the HR-negative group.

A fully cross-validated classification analysis was performed to determine whether EA-CSF volume at 6 months could correctly distinguish which infants would be classified with an ASD diagnosis at 24 months. The objective of the prediction analysis was to extend beyond what can be concluded by the mixed effects model by determining the specificity and sensitivity of EA-CSF volume at the single earliest time point (i.e., 6 months of age) to separate HR-ASD from HR-negative infants. Only HR infants were included in the prediction model to distinguish HR-ASD infants from HR-negative infants using data at 6 months only. To remain consistent with the primary mixed effects model described above, the same covariates were included in the prediction model (sex, age, and TCV at 6 months). A 25-fold cross-validation was implemented where 1/25th of the IBIS sample (4%) was left out of the prediction model, the model was built ("trained") on the remaining 96% subjects, and then was used to independently predict the 4% – this was repeated 25 times until the entire sample had been predicted *(via a supervised machine learning classification with a balance-boosted trees ensemble algorithm using RUSBoost trees)* (53). The overall accuracy of the prediction model was reported as the area under the receiver operating characteristic curve (AUC), with the corresponding sensitivity and specificity threshold determined by the receiver operating characteristic (ROC) curve. 95% confidence intervals for the reported proportions (sensitivity, specificity) were calculated according to the efficient-score method and corrected for continuity (54). We also performed an out-of-sample validation by using this model built on the IBIS data and testing it on the dataset from the original Shen et al. (2013) paper on EA-CSF.

Regression analyses (both ordinary least squares and robust regression *ROBUSTREG* in SAS) were generated to test the *a priori* hypothesis that EA-CSF at 6 months would be associated with gross motor ability measured at 6 months within the ASD group. Clinical variables of interest included Mullen subscale and Vineland motor scores.

Group differences in LV volume were tested using the same mixed effects model described above, and linear regression was used to test for associations and group interactions between LV and EA-CSF at each time point. All tests were two-tailed with α = 0.05. All analyses were performed using SAS JMP software (SAS Institute, Cary, NC).

RESULTS

There were no significant group differences in demographic variables (race/ethnicity, maternal education level, or family income) (22). There were no significant group differences in age at each MRI time point (Table 1). As expected, at 24 months the HR-ASD group had significantly lower cognitive ability on the Mullen Early Learning Composite, and higher ASD symptom scores on the ADOS (total scores for Social Affect + Repetitive, Restricted Behaviors), compared to the two comparison groups (Table 1).

Extra-axial CSF volume

There was a significant negative effect of subject age (age: $\beta=-3.38$, $F_{1,483}=50.97$, $p<0.0001$; age^2: $\beta=0.07$, $F_{1,486}=40.02$, $p<0.0001$) on EA-CSF volume. Total cerebral volume was significantly associated with EA-CSF ($\beta=0.05$, $F_{1,264}=28.51$, $p<0.0001$). There was no significant main effect of sex or group x sex interaction ($F_{1,277}=0.08$, $p=0.78$; group $\times$ sex: $F_{2,398}=2.46$, $p=0.09$), indicating that EA-CSF did not differ significantly between male and female infants after controlling for age and TCV. There were no differences in EA-CSF by scan site ($F_{3,272}=0.11$, $p=0.96$).

High-risk infants who were later diagnosed with ASD had increased EA-CSF at 6 months, which remained significantly elevated through 24 months. Specifically, there was a significant main effect of group ($\beta=16.01$, $F_{2,397}=6.04$, $p=.0026$), and no significant group $\times$ age interactions (group $\times$ age: $F_{2,294}=2.40$, $p=0.09$; group $\times$ age^2: $F_{2,274}=1.87$, $p=0.16$), indicating that the increase in EA-CSF in the HR-ASD group relative to non-ASD groups was consistent over the interval studied (covariates included age, age^2, TCV, sex, site). Direct group comparisons and inspection of the model parameter estimates indicated that, on average across the study period, the HR-ASD group had 12.20 cm^3 more EA-CSF than the HR-negative group ($\beta=12.20$; se=3.96; $t_{397}=3.08$; $p=.002$) and 12.14 cm^3 more EA-CSF than the LR-negative group ($\beta=12.14$; se=4.10; $t_{397}=2.96$; $p=.003$), after controlling for age, age^2, TCV, sex, and site. There were no differences between the HR-negative and LR-negative group ($\beta=.06$; se=2.11; $t_{397}=.03$; $p=.98$). Figure 2 depicts an example of a LR infant with a normal level of EA-CSF, compared to a HR infant who had increased EA-CSF at 6, 12, and 24 months and was diagnosed with ASD at 24 months. (See Supplemental Figure S1 for example images from representative infants in each group who have EA-CSF volumes that are equal to their group's average.) Figure 3 illustrates the group trajectories of EA-CSF from 6–24 months, with percent differences between model-adjusted group means and Cohen's *d* effect size (relative to the HR-negative group) at each time point. (Individual trajectories are shown in supplemental Figure S2.)

EA-CSF and Subgroups of Autism Severity

To examine whether subgroups with different levels of autism severity were associated with differences in EA-CSF volume, the ASD group was stratified into subgroups according to well-established, empirically derived categories on the ADOS that index severity of autism symptoms (9; 29). There was a significant main effect of group (β=25.77, $F_{3,416}$=4.99, p=.002) with the infants with more severe autistic behaviors (ASD-High) having significantly greater EA-CSF volume at all time points compared to each of the other groups, including the ASD-Moderate, HR-negative, and LR-negative groups (covariates: age, age^2, TCV, sex, site). Direct group comparisons and inspection of the model parameter estimates revealed that, on average across the study period, the ASD-High group had significantly greater EA-CSF than the ASD-Moderate group (β=19.31; se=8.19; t_{416}=2.36; p=.02), HR-negative group (β=22.59; se=6.02; t_{416}=3.75; p=.0002), and LR-negative group (β=22.61; se=6.11; t_{416}=3.70; p=.0002), controlling for age, age^2, TCV, sex, and site. The ASD-moderate group did not differ significantly from the HR-negative (β=3.28; se=5.38; t_{416}=.61; p=.54) and LR-negative groups (β=3.30; se=5.46; t_{416}=.60; p=.55) in EA-CSF volume. Total cerebral volume was significantly associated with EA-CSF (β=0.05, $F_{1,263}$=29.44, p<0.0001). There was no significant main effect of sex ($F_{1,286}$=.34, p=0.56), indicating that EA-CSF volume did not differ between male and female infants after controlling for age and TCV. There was a significant group × sex interaction ($F_{3,397}$=2.87, p=0.04) with the small number of female infants in the ASD-High group (n=2 of the 23 in the subgroup) having higher EA-CSF volume on average (t_{397}=2.14; p=.03) compared to the male infants in the ASD-High group (n=21). Figure 4 illustrates the group trajectories of EA-CSF from 6–24 months, with percent differences between model-adjusted group means and Cohen's *d* effect size (relative to the HR-negative group) at each time point. (Individual trajectories are shown in supplemental Figure S3.)

Does EA-CSF at 6 Months Predict Autism Diagnosis at 24 Months?

A fully cross-validated prediction analysis was performed to test whether EA-CSF volume at 6 months could accurately classify which HR infants would be diagnosed as ASD vs. negative for ASD at 24 months. EA-CSF volume at 6 months classified HR-ASD infants at an overall accuracy of 69% (AUC=0.69), with corresponding sensitivity of 66% (95% CI: 50.6–78.7) and specificity of 68% (95% CI: 60.3–74.6). We performed an out-of-sample validation on this model, which was built on the current dataset, by testing it on the dataset from the original Shen et al. (2013) paper on EA-CSF. The prediction model applied to the 2013 dataset yielded similar accuracy: overall accuracy of 72%, sensitivity of 80% (95% CI: 44.2–96.5), and specificity of 67% (95% CI: 38.7–87.0).

EA-CSF Association with Early Motor Skills within the ASD group

Given that motor symptoms are an early emerging feature in infants who develop ASD (7; 9; 37) and that increased EA-CSF had previously been associated with motor impairments (13–16), we hypothesized that EA-CSF in early infancy would be related to early motor function at 6 months within the HR-ASD group. EA-CSF volume at 6 months was significantly correlated with poorer motor skills at 6 months in two measures: the direct examination Mullen gross motor subscale ($F_{1,45}$=11.72, p=.0013; R^2=.207; r = −0.46; robust regression:

χ^2= 12.55, df=1, p=.0004) and the parent-report Vineland motor skills sub scale ($F_{1,45}$=7.28, p=.0098; R^2=.134; r = −0.37; robust regression: χ^2=11.02, df=1, p=.0009) (Figs. 5A–5B). There were no significant correlations between EA-CSF and other Mullen subscales at 6 months: receptive language ($F_{1,45}$=.99, p=.3253; R^2=.022; r = −0.15; robust: χ^2=.56, df=1, p=.4540), expressive language ($F_{1,45}$=1.83, p=.1827; R^2=.039; r = 0.20; robust: χ^2=1.21, df=1, p=.2708), visual reception ($F_{1,45}$=3.17, p=.0818; R^2=.066; r = −0.26; robust: χ^2=1.56, df=1, p=.2115), or fine motor ($F_{1,45}$=3.19, p=.0810; R^2=.066; r = −0.26; robust: χ^2=2.54, df=1, p=.1109).

Relationship to Lateral Ventricle Volume

There were no significant group differences in LV volume ($F_{2,383}$=1.98, p=0.14) or group × age interaction ($F_{2,266}$=1.24, p=0.29). There was a significant negative effect of age on LV volume (β=−0.37, $F_{1,602}$=11.72, p=0.0007). Total cerebral volume was significantly associated with LV volume (β=0.02, $F_{1,496}$=44.34, p<0.0001). There was no significant main effect of sex ($F_{1,335}$=1.43, p=0.23) or group × sex interaction ($F_{2,331}$=0.20, p=0.82), indicating that LV volume did not differ between male and female infants after controlling for age and TCV. There were no differences in LV by scan site ($F_{3,320}$=0.28, p=0.84).

The HR-ASD group did not show a significant correlation between LV and EA-CSF volume at 6 months ($F_{1,44}$=1.34, p=.25; R^2=.029; r = 0.17), 12 months ($F_{1,28}$=1.35, p=.25; R^2=.046; r = 0.21), or 24 months ($F_{1,30}$=2.54, p=.12; R^2=.078; r=0.28), and the relationship between LV and EA-CSF did not differ significantly between groups at any age (group interaction at 6 months: $F_{2,335}$=1.50, p=0.23; 12 months: $F_{2,247}$=0.004, p=0.99; 24 months: $F_{2,201}$=0.29, p=0.75).

DISCUSSION

In this study, high-risk infants diagnosed with ASD at 24 months had significantly increased EA-CSF volume from 6 months through 24 months of age. Differences in EA-CSF volume were not accounted for by brain size and were observed in the absence of enlarged ventricles. Because of the relatively large sample of infants at risk, it was possible to assess whether EA-CSF volume differed among subgroups defined by autism symptom severity. Increased EA-CSF volume was more pronounced from 6–24 months in the subgroup of infants who had the most severe autistic behaviors at 24 months. These results confirm and extend the findings of Shen *et al.* (1) in an independent sample of infants recruited from institutions across the United States, confirming that increased extra-axial CSF is a replicable brain anomaly that is detectable as early as 6 months of age and remains elevated through 24 months in high-risk infants who go on to develop ASD.

Although increased extra-axial CSF has been observed in the first year of life in the clinical radiology literature (10–12), excess CSF is thought to decrease to normal levels in the second year. However, longitudinal follow-up with imaging and behavioral assessment is rarely conducted (for review, see (38). There have been a few reports linking increased extra-axial CSF with early motor delay (13–16), and early motor deficits have been widely reported in HR infants who later attain a diagnosis of ASD (7; 9; 37; 52). In the present study, increased EA-CSF volume at 6 months was associated with poorer motor skills on

both direct examination and parent report, supporting the hypothesis that increased EA-CSF may affect motor development during the prodromal period in autism, before behaviors diagnostic of ASD typically arise.

The question arises as to whether persistently increased EA-CSF indicates a role for abnormal CSF circulation in the pathogenesis of ASD or, alternatively, is epiphenomenal and indicative of some other underlying process. Excessive CSF in the subarachnoid space, in the absence of enlarged ventricles, could be an indication of impaired CSF circulation and absorption, which can lead to altered concentration of neural growth factors and potentially harmful metabolites that have a pathological effect on normal brain development (21). The recent discoveries of the glymphatic and meningeal lymphatic systems of the brain both highlight the importance of proper CSF circulation and absorption to clear metabolic byproducts from the brain (20; 39; 40). CSF circulation is responsible for the removal of potentially neurotoxic waste products and inflammatory cytokines that accumulate in the brain (21). For example, amyloid-beta (Aβ) is a neurotoxic protein that is cleared from the interstitial and subarachnoid space during CSF circulation and absorption (39). Amyloid-beta has been found to be elevated in blood, peripheral CSF, and post mortem human brain tissue in individuals with ASD (41–45). Evidence of increased Aβ levels in ASD has been linked to sleep disturbances (which can disrupt clearance of Aβ) (20), seizures, and deficits in motor and cognitive function (41; 42). Altered CSF circulation results in an accumulation of metabolic byproducts and an imbalance of inflammatory cytokines and growth factors (20; 21), and altered composition of CSF has been shown to have a pathological effect on human brain development (18; 19). Future studies, perhaps in animal models of ASD, will need to be carried out to evaluate both the underlying causes of persistently increased EA-CSF and the potential deleterious effects on brain development. While EA-CSF may have a pathogenic role in the etiology of ASD, it is also quite possible that increased EA-CSF is a marker of some other underlying process that may indicate a more general risk for altered neurodevelopment.

There has been recent emphasis placed on the importance of finding biological markers to aid in evaluating early risk for neurodevelopmental and other brain-based, behavioral disorders (46–48). A major obstacle in this pursuit is the lack of replication of putative biomarkers and/or small sample sizes (for review, see (49). The current study addressed both concerns, confirming the initial findings in an independent sample roughly seven times larger than the first sample. Reproducibility is rare in biomedical research (50), particularly in neuroscience (51) and autism (49). This study not only confirms findings in an independent sample, but it employed a different image acquisition protocol, multiple scanners and study sites, and different (automated) image analysis procedures. The results were strikingly similar across both studies, suggesting that the findings are robust. This view will be further supported if future studies on independent populations come to similar conclusions.

We developed a fully automated method to quantify EA-CSF volume, and the current findings suggest that increased EA-CSF is an observable brain anomaly that potentially could be quantified using different structural MRI platforms available to clinical radiologists. Future studies will determine how these quantitative measurements correspond

to qualitative ratings from radiological assessments (22). Before quantification of EA-CSF could be useful in a clinical setting, the sensitivity and specificity of predicting autism must be established. Increased EA-CSF volume at 6 months had 69% accuracy in predicting autism at 24 months. These prediction metrics were similar to those found in the out-of-sample validation of the previous sample (1), which supports the predictive validity of this finding. However, the results of the prediction model are not yet strong enough as a stand-alone marker to be clinically useful in predicting individual outcomes. Furthermore, the specificity of EA-CSF for ASD needs to be evaluated, as it is possible that extra-axial CSF may be a more general marker for altered neurodevelopment. Thus, future studies are needed to evaluate whether infants with other neurodevelopmental disorders also show increased EA-CSF during the first two years of life.

This study raises a number of questions that need to be addressed in order to evaluate what, if any, are the potential clinical implications of these findings. Is increased EA-CSF associated with ASD only in children at high familial risk, or would it be found more generally in other children who develop ASD? Is it specific to autism, or is it present in children who develop other neurodevelopmental disorders? What leads to increased EA-CSF? Is it associated with immunological insults, and are there genetic underpinnings? The answers to these questions would contribute to decisions as to whether the presence of increased extra-axial CSF should be assessed and monitored routinely in infants at risk for ASD. Though the current clinical view is that early increased extra-axial CSF is commonly benign and without long-term consequences, this should be re-evaluated in infants at risk for ASD in light of the findings of this study and the predecessor study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

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ADOS	Autism Diagnostic Observation Schedule
ADI-R	Autism Diagnostic Interview -Revised
ASD	autism spectrum disorder
AUC	area under the receiver operating characteristic curve
EA-CSF	extra-axial cerebrospinal fluid
HR	high familial risk for ASD
HR-ASD	high risk infants later diagnosed with ASD
HR-negative	high risk infants who do not develop ASD
LR	low familial risk for ASD
LR-negative	low risk infants who do not develop ASD
LV	lateral ventricles
ROC	Receiver operating characteristic
TCV	total cerebral volume

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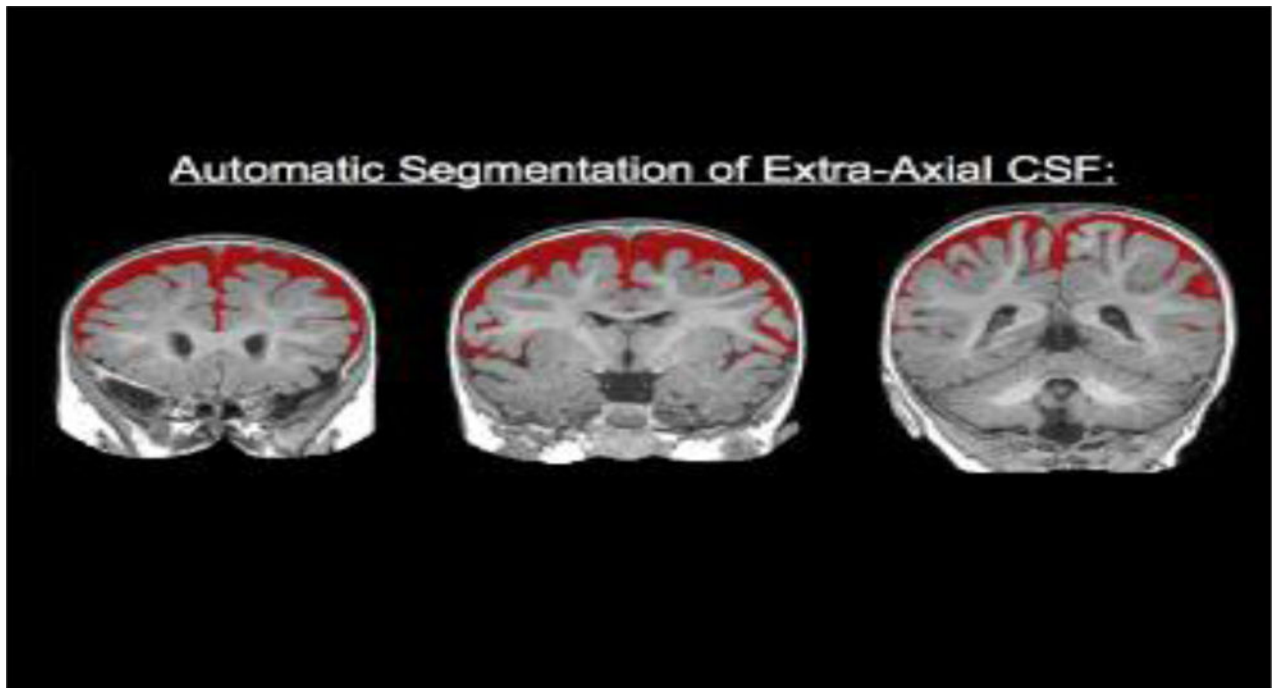
Figure 1. Automated quantification of extra-axial CSF
T1- and T2-weighted images were acquired from each participant and used to segment the cerebrospinal fluid in the subarachnoid space between the dura and cortical surface, dorsal to the horizontal plane of the anterior-posterior commissure.
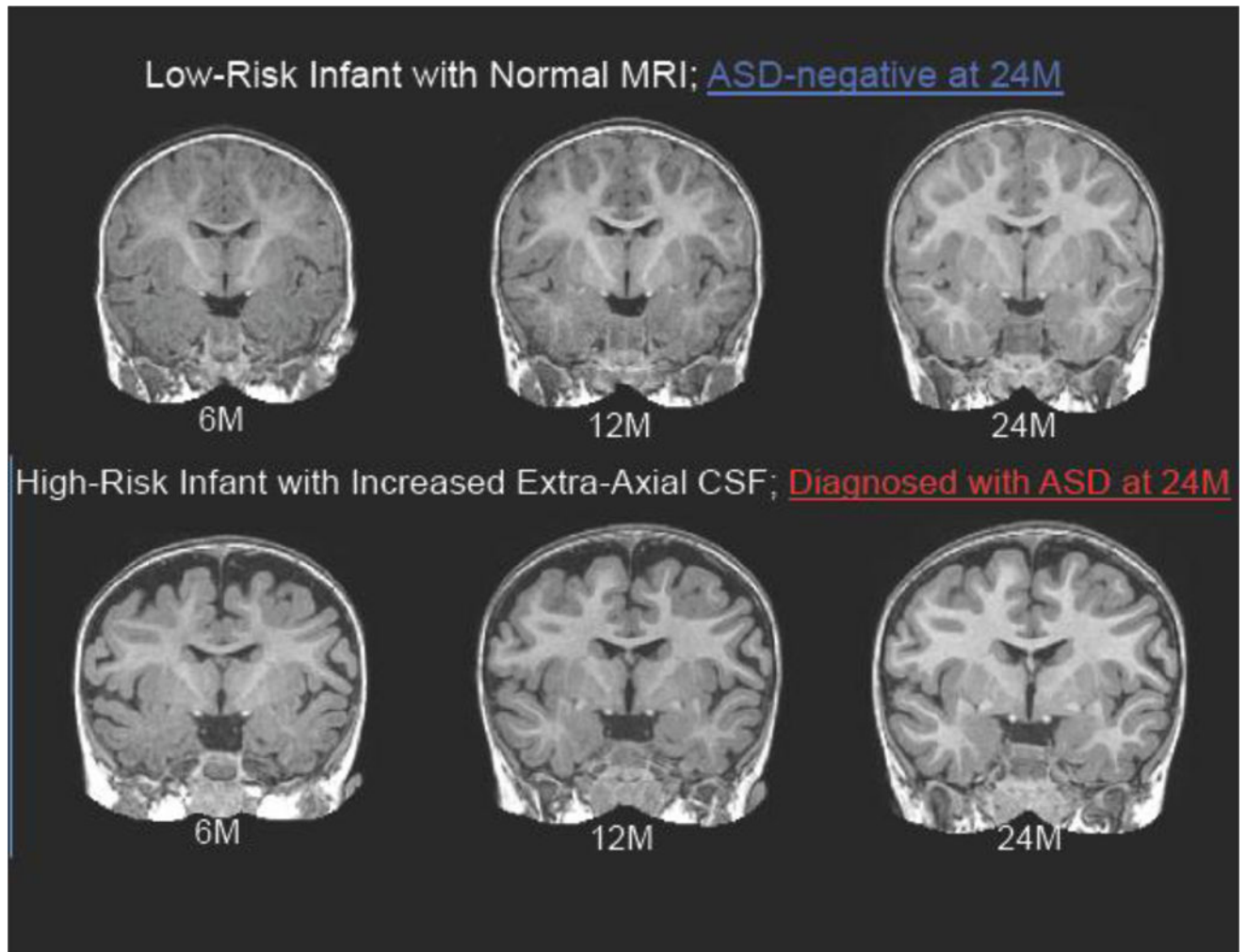
Figure 2. Example brain images indicating the presence of increased extra-axial CSF
(A) T1-weighted coronal images of a low-risk infant with normal MRI at 6, 12, and 24 months. (B) T1-weighted coronal images of a high-risk infant with increased extra-axial CSF at 6, 12, and 24 months. This child was diagnosed with ASD at 24 months.
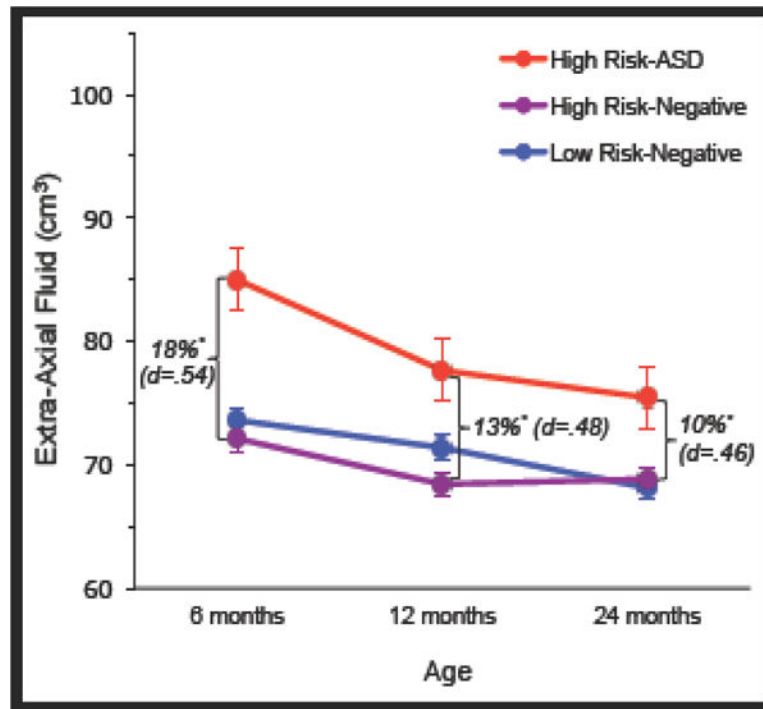
Figure 3. Infants later diagnosed with ASD had increased extra-axial CSF by 6 months, which remained significantly elevated through 24 months

Note: LS means are adjusted for covariates in model [age, sex, total cerebral volume, scan site]. Error bars = ±1 SEM. *p<0.005 vs. HR-negative and vs. LR-negative. Percent differences are in relation to the HR-negative group (Cohen's d).
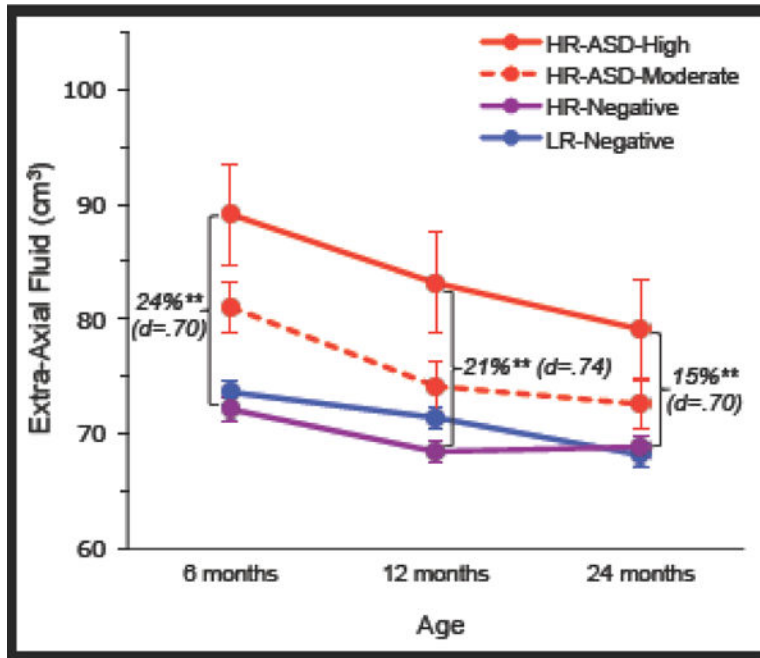
Figure 4. ASD subgroup with more severe autism symptoms had a greater increase of extra-axial CSF throughout 6-24 months compared to all other groups

The ASD group was stratified into subgroups according to empirically derived categories on the ADOS. The ASD subgroup with more severe autism symptoms (HR-ASD-High) had a more pronounced increase in extra-axial CSF. *Note: LS means are adjusted for covariates in model [age, sex, total cerebral volume, scan site]. Error bars = ±1 SEM. **p<0.0005 vs. HR-negative and vs. LR-negative, p<0.05 vs. HR-ASD-Moderate. Percent differences are in relation to the HR-negative group (Cohen's d).*
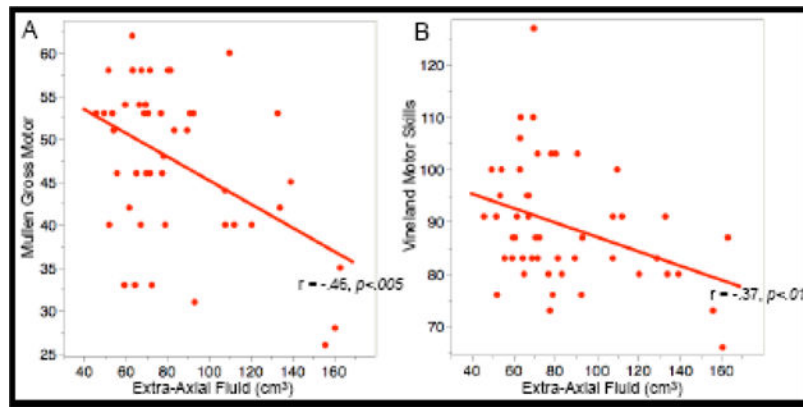
Figure 5. Extra-axial CSF is significantly correlated with poorer motor skills in the ASD group
Across the entire HR-ASD group, extra-axial CSF volume at 6 months of age was negatively correlated with motor scores at 6 months on the (A) direct examination Mullen gross motor subscale *(standardized norm of M[SD] = 50[10])*; and (B) parent-report Vineland motor subscale *(standardized norm of M[SD] = 100[15])*.

Table 1

Participant characteristics by diagnostic outcome group

	Mean (SD)			
	High Risk-ASD	**High Risk-Negative**	**Low Risk-Negative**	**Test statistic**[a]
N	47	174	122	
Sex	42 M; 5 F	95 M; 79 F	76 M; 46 F	$X^2(2)$=21.94, p=1.72 × 10^{-5}
Age at 1st MRI (mo.)	6.6 (.7)	6.6 (.7)	6.7 (.7)	$F_{2,340}$=0.57, p=.57
Age at 2nd MRI (mo.)	12.8 (.7)	12.6 (.6)	12.7 (.8)	$F_{2,251}$=2.14, p=.12
Age at 3rd MRI (mo.)	24.7 (.7)	24.8 (.9)	24.7 (.8)	$F_{2,204}$=0.35, p=.71
Mullen Early Learning Composite (at 24 mos.)	77.8 (18.6)	102.6 (15.9)	109.8 (13.4)	$F_{2,204}$=46.05, p=3.13 × 10^{-17} 2,204
ADOS Total (at 24 mos.) (Social Affect+RRB)	14.2 (5.5)	2.7 (2.3)	2.5 (2.3)	$F_{2,204}$=48.51, p=5.83 × 10^{-18} 2,204

[a]Test statistic, degrees of freedom, and p-value of omnibus ANOVA (Age, Mullen, ADOS) and Chi-square test (sex)

Table 2

Number of MRI scans at each time point

No. of scans at each time point	High-risk ASD	High-Risk Negative	Low-Risk Negative	Total scans at each time point
6 months	47	174	122	**343**
12 months	31	134	89	**254**
24 months	32	111	64	**207**
Total N	**110**	**419**	**275**	**804**