Archival Report

Biological Psychiatry

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 autism spectrum disorder (ASD) had increased cerebrospinal fluid (CSF) in the subarachnoid space (i.e., extra-axial CSF) from 6 to 24 months of age. We attempted to confirm and extend this finding in a larger independent sample.

METHODS: A longitudinal magnetic resonance imaging study of infants at risk for ASD was carried out on 343 infants, who underwent neuroimaging at 6, 12, and 24 months. Of these infants, 221 were at high risk for ASD because of an older sibling with ASD, and 122 were at low risk with no family history of ASD. A total of 47 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 with 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 to 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: Autism, Brain development, CSF, Extra-axial fluid, Infancy, MRI

http://dx.doi.org/10.1016/j.biopsych.2017.02.1095

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 (1). ASD affects about 1% to 2% of children worldwide (2–4). 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 (5). There are currently no biomarkers detectable during 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 during the latter part of the first and second years of life (6–8).

Shen *et al.* (9) reported that high-risk infants who later developed ASD had increased extra-axial cerebrospinal fluid (EA-CSF) volume from 6 to 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

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 ISSN: 0006-3223

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) a multisite 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.* (9), we hypothesized that 1) HR infants later diagnosed with ASD (HR-ASD) would show increased EA-CSF volume at 6 months compared with 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) (22). HR infants had an older sibling with a clinical diagnosis of ASD, corroborated by the Autism Diagnostic Interview–Revised (24). LR infants had a typically developing older sibling and no first- or second-degree relatives with intellectual/psychiatric disorders (8). See Supplement for full inclusion/exclusion criteria. Parents provided informed consent, and the institutional review board at each site approved the research protocol.

Assessment

Infants were assessed at 6, 12, and 24 months with magnetic resonance imaging (MRI) scans 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–Generic [ADOS (28)] were administered to all participants at 24 months. The Autism Diagnostic Interview–Revised 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), Autism Diagnostic Interview–Revised, and other behavioral measures. Further details on the assessment and diagnostic procedures can be found in Estes *et al.* (8). A small number of LR infants who met criteria for ASD ($n = 3$) were excluded from the analysis because they were too few in number 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 they 1) had a successful, high-quality MRI at least at the initial 6-month visit and 2) were assessed for an ASD diagnosis at the 24-month visit. A total of 343 infants (221 HR and 122 LR) met these criteria and were included in the analysis, yielding three outcome groups: 1) HR-ASD ($n = 47$; 42 male and 5 female), 2) HR-negative ($n = 174$; 95 male and 79 female), and 3) LR-negative ($n = 122$; 76 male and 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 *et al.* 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 the threshold (ASD-Moderate subgroup; $n = 24$). This approach is consistent with previous publications on this sample (8).

MRI Acquisition

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

Table 1. Participant Characteristics by Diagnostic Outcome Group

	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	$\chi^2_2 = 21.94$, $p = 1.72 \times 10^{-5}$
Age at First MRI, Months	6.6 (0.7)	6.6 (0.7)	6.7 (0.7)	$F_{2,340} = 0.57$, $p = .57$
Age at Second MRI, Months	12.8 (0.7)	12.6 (0.6)	12.7 (0.8)	$F_{2,251} = 2.14$, $p = .12$
Age at Third MRI, Months	24.7 (0.7)	24.8 (0.9)	24.7 (0.8)	$F_{2,204} = 0.35$, $p = .71$
Mullen Early Learning Composite at 24 Months	77.8 (18.6)	102.6 (15.9)	109.8 (13.4)	$F_{2,204} = 46.05$, $p = 3.13 \times 10^{-17}$
ADOS Total at 24 Months (Social Affect + Restricted Repetitive Behaviors)	14.2 (5.5)	2.7 (2.3)	2.5 (2.3)	$F_{2,204} = 48.51$, $p = 5.83 \times 10^{-18}$

Data represent mean (SD) or *n*.
ADOS, Autism Diagnostic Observation Schedule–Generic; ASD, autism spectrum disorder; F, female; M, male; MRI, magnetic resonance imaging.
[a]Test statistic, degrees of freedom, and *p* value of omnibus analysis of variance (age, Mullen, and ADOS) and chi-square test (sex).