Published in final edited form as:

Biol Psychiatry. 2013 October 15; 74(8): 563–575. doi:10.1016/j.biopsych.2013.03.022.

COMPARED TO WHAT? EARLY BRAIN OVERGROWTH IN AUTISM AND THE PERILS OF POPULATION NORMS

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Abstract

Background—Early brain overgrowth (EBO) in autism spectrum disorder (ASD) is amongst the best-replicated biological associations in psychiatry. Most positive reports have compared head circumference (HC) in ASD (an excellent proxy for early brain size) with well-known reference norms. We sought to reappraise evidence for the EBO hypothesis given (i) the recent proliferation of longitudinal HC studies in ASD, and (ii) emerging reports that several of the reference norms used to define EBO in ASD may be biased towards detecting HC overgrowth in contemporary samples of healthy children.

Methods—(1) Systematic review of all published HC studies in children with ASD. (2) Comparison of 330 longitudinally gathered HC measures between birth and 18 months from male children with autism (n=35) and typically developing controls (n=22).

Results—In systematic review, comparisons with locally recruited controls were significantly less likely to identify EBO in ASD than norm-based studies ($p < 0.006$). Through systematic review and analysis of new data we replicate seminal reports of EBO in ASD relative to classical HC norms, but show that this overgrowth relative to norms is mimicked by patterns of HC growth age in a large contemporary community-based sample of US children (n~75,000). Controlling for known HC norm biases leaves inconsistent support for a subtle, later-emerging and sub-group specific pattern of EBO in clinically-ascertained ASD vs. community controls.

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FINANCIAL DISCLOSURES

All authors report no biomedical financial interests or potential conflicts of interest.

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Conclusions—The best-replicated aspects of EBO reflect generalizable HC norm biases rather than disease-specific biomarkers. The potential HC norm biases we detail are not specific to ASD research, but apply throughout clinical and academic medicine.

Keywords

Autism; Head Circumference; CDC; WHO; Systematic Review; Bias

INTRODUCTION

An atypical pattern of brain growth during early postnatal life was first reported in children with autism over a decade ago(1). Since then, numerous studies have used head circumference (HC) measures, or *in vivo* structural magnetic resonance imaging estimates of brain size, to test for early brain overgrowth (EBO) in children with autism spectrum disorder (ASD).

The EBO hypothesis, which states that ASD is associated with an abnormal acceleration of brain growth within the first two years of life(2), has received considerable empirical support - leading to the speculation that EBO might be a potential biomarker for ASD(3). The influence of EBO reports on ASD research is evidenced by recent use of the link between brain enlargement and ASD to validate or interpret (i) animal models for putative genetic(4), and epigenetic(5) risk mechanisms in ASD, (ii) studies of postmortem brain tissue from individuals with ASD(6,7), (iii) reported associations between a given genetic variant and risk for ASD(8), and (iv) in-vivo neuroimaging and electrophysiological studies of altered brain connectivity in ASD(9–11). Two recent developments urge re-appraisal of the evidence base for EBO in ASD however.

First, several new longitudinal studies of early brain growth in ASD have become available since the topic last underwent systematic review(12). Longitudinal data are critical for testing the EBO hypothesis, which hinges on the presence of an atypical pattern of brain size *change* in ASD(13). Currently, the largest available body of evidence regarding early brain growth in ASD comes from studies of HC, which serves as an excellent proxy for brain size in infants and preschool-aged children(14,15) and provides cost-effective access to large bodies of retrospective longitudinal data about brain growth in ASD during the first years of postnatal life. There are now 11 longitudinal HC studies of brain growth in ASD within the hypothesized phase of EBO(15–25), which together provide 17 times (~3000:180) more observations than the two existent longitudinal structural neuroimaging studies of preschoolers with ASD(26,27). As 10 of these 11 longitudinal HC studies have been published since the topic of EBO was last systematically reviewed(12), there is a pressing need to formally integrate the now much expanded evidence base regarding patterns of early brain growth in ASD. Such integration could also help clarify recently posed questions regarding the selectivity of EBO for certain ASD subgroups [e.g as defined by sex or clinical status(22)], and the extent to which EBO in ASD is part of more generalized somatic overgrowth(21).

The second recent development that could significantly modify our understanding of EBO in ASD comes from multiple studies outside the field of ASD, which report discrepancies

between HC growth reference norms commonly used to test the EBO hypothesis in ASD, and contemporary patterns of HC growth(28–33). The best replicated of these discrepancies concerns Center for Disease Control (CDC) norms(34): to date, five large independent contemporary samples of healthy children have been reported to show trajectories of HC growth during the first year of life that are abnormally accelerated relative to the CDC norms(29–33). This discrepancy is strikingly similar to the pattern of HC overgrowth relative to CDC norms which constitutes the principal finding in five of the most commonly cited sources of empirical support for the EBO hypothesis in ASD(15,16,19,20,35). This convergence raises a critical question - might reports of HC growth in ASD that diverge from CDC norms reflect a systematic bias in CDC norms rather than any specific association between ASD and an abnormal acceleration of early brain growth? Concerns also arise regarding potential biases in other popular HC norms given robust and convergent evidence that the tempo of human HC growth can show robust inter-generational change, and that such secular changes in HC growth can emerge within time-frames that have commonly separated the construction of these HC norms and their application in ASD research[United Kingdom(28,36), China(37), Netherlands(38), Finland(32), Japan(39), Korea(40)]. The influence of HC norm use on early brain growth findings in ASD is yet to be systematically assessed however, despite carrying significant consequences for the EBO hypothesis in ASD, and more generally, for the application of population reference norms in clinical and academic medicine.

In the present report, we reappraise evidence for EBO in ASD through an updated systematic review of all HC studies in children with ASD and analysis of HC data from a recently assembled cohort of children with autism and typically developing controls(41). Our study builds on the last systematic review of the EBO hypothesis(12) in two main ways. First, we incorporate the expanded body of evidence base regarding EBO in ASD, which has been enriched by multiple longitudinal studies capable of quantifying those changes brain size that are so critical to the EBO hypothesis. Second, we provide a detailed methodological annotation of published research, so that available evidence for and against the EBO hypothesis can be considered in light of pertinent study features including (i) age-range (ii) temporal density of measures used to generate group HC estimates, (iii) extent of control for potential HC modifiers such as body size, sex, and ethnicity and (iv) source of control data with which ASD HC measures are contrasted. The relationship between control group selection and HC findings in ASD is a major empirical focus in both our systematic review and analysis of new HC data, which we address by (i) comparing EBO findings in ASD studies that rely exclusively on HC norms with ASD studies that include locally recruited control children as a comparison group, (ii) comparing EBO findings in ASD across multiple HC reference norms, and (iii) using the common frame of CDC norms to integrate reported patterns of early HC growth in children with ASD and locally recruited controls, and then compare these trajectories with the largest (~400,000 HC measures on 75,000 children ages 0–18 months) and most current community-based sample of US children [“Primary Care Network” (PCN)] norms(30)].

METHODS

Systematic Review

Three authors (A.R, G.W, L.A) independently carried out an electronic literature search [PUBMED, EMBASE, start of records until June 30th 2012], and manual bibliography search to identify all available studies of HC in children with ASD. Electronic literature search terms included: “AUT*”, “ASPERGER*”, “ASD”, “PERVASIVE DEVELOPMENTAL DISORDER”, “PDD”, “HEAD CIRCUMFERENCE”, “OFC”, and “OVERGROWTH”. For a study to enter our initial pool, it had to include (i) children with ASD under age 5 years, or an ASD group with mean age 10 years, and (ii) a comparison of ASD HC values with those in control children or HC norms. Our requirements regarding study age were designed to capture all studies including children. Of the 43 studies thus identified (Figure S1 in Supplement 1), 34 survived exclusion criteria (Table 1). Three authors (A.R, G.W, L.A) independently abstracted data from all 34 studies using a common set of rules (Table S2 in Supplement 2), and any inter-rater inconsistencies were resolved by consensus.

To graphically integrate reported early-life HC trajectories for children with ASD and controls (e.g as in Figure 2) we averaged reported mean group HC estimates across studies at standard pediatric health surveillance check-points (birth, 2, 4, 6, 9, 12, 15, and 18 months). Whenever averaging HC values (e.g HC centile or macrocephaly rate) across studies, each reported HC value was weighted by the number of HC observations-per-month upon which it was based. This weighting is needed to reflect the fact that 100 children aged 10 months +/- 2 weeks provide a more valid estimate of mean HC at 10 months than 100 children aged 10 months +/- 4 months.

Chi-squared tests were used to quantify the relationship between cross-sectional study outcome (report of statistically significant evidence of HC enlargement in ASD/vs. report of no such evidence) and use of HC growth norms (HC norms vs recruited controls) (see text in Supplement 1 for further details). This approach was adopted after testing for, and ruling out the presence of an association between ASD sample size and study outcome ($p=0.5$ for macrocephaly reports, $p=0.3$ for HC centile reports).

Analysis of New HC Data

Participants—We included a total of 57 males, who were enrolled at approximately 4 years of age and comprised 22 locally recruited typically developing controls, and 35 clinically-ascertained children with ASD who met DSM-IV diagnostic criteria for Autistic Disorder. Full details of procedures for participant recruitment, screening, medical investigation and cognitive assessment are provided in Supplement 1. In all cases, written informed consent was obtained from the participant’s parent(s). A NIH Institutional Review Board approved this study.

Head Circumference Data—Head circumference data were retrospectively gathered for all participants from medical records. As expected, HC measures were clustered at birth, 2, 4, 6, 9, 12, 15, and 18 months of age [as per American Academy of Pediatrics

Recommendations for Preventative Pediatric Health Care(42)]. Our sample included a total of 330 HC measures (201 ASD, 129 controls). Head circumference values were analyzed in both their raw form and after conversion to age and sex-normed HC centile using CDC(34), WHO(43) and PCN(30) norms.

We note that the number of participants and overall HC measurements in our sample is smaller than some prior reports (see Table 1) and therefore less powered to identify statistically significant group differences in HC. However, in the context of our systematic-review/meta-analysis of prior data, the main purpose of analyzing our own raw HC data was to (i) determine if our newly-derived HC trajectories would independently replicate our systematic review findings, (ii) allow illustration of different HC norm effects on the same raw data, and (iii) allow illustration of the distributional properties that arise when normally distributed raw HC data are converted to centile.

Statistics—After assessing if HC indices at each age-point violated the assumption of normality (Shapiro-Wilk test), parametric (Student's T) or non-parametric (Wilcox Signed Rank) tests were applied as appropriate to carry out serial cross-sectional comparisons of (i) children with ASD vs. controls for both raw HC and HC centile, and (ii) HC centile in children with ASD and controls vs. the norm-predicted HC centile value of 50.

Longitudinal analysis of HC data was first conducted by comparing changes in HC between adjacent age-points using the Mann Whitney-U tests (given non-normality of HC change according to Shapiro-Wilks test). Next, for the 44 participants (26 ASD, 18 controls) with at least 5 HC measures, we used non-linear mixed models (nlme package in R) to model HC growth according to the following asymptotic regression equation:

$$HC = \varphi_1 + \varphi_2(e^{(\varphi_3 * t)})$$

This growth curve was chosen for consistency with prior non-linear modeling of HC in ASD(17,24). Sequential model fitting with F-test comparison between models was used to specify random effects, and determine if ASD and control groups showed significant differences in HC growth (either for individual parameters or across all parameters).

FINDINGS: SYSTEMATIC REVIEW

Overview

Table 1 lists the 34 studies identified by our systematic review(15–22,24,25,35,44–66), according to publication date and study characteristics.

Survey of Normative Data Used to Test EBO Hypothesis in ASD

Of the 34 studies identified, twenty-one studies made exclusive use of HC norms as comparison data when testing the EBO hypothesis, eight studies used both norms and locally recruited controls, and five studies made exclusive use of locally recruited controls. Head circumference norms used in tests of the EBO hypothesis vary greatly in the historical, geographical, and ethnic properties of the underlying samples they are based upon (Table 2).

While cross-sectional HC studies in ASD have drawn on a diverse pool of HC norms, 88% of all longitudinal studies have relied on CDC norms.

Cross-sectional Studies

Thirty-two (94%) of the 34 studies identified by systematic review included a cross-sectional comparison of HC between participants with ASD and previously published norms or HC in locally-recruited controls. Cross-sectional comparisons between HC in children with ASD and locally recruited controls more often failed to find evidence for EBO than when comparisons are based on previously published HC norms (Chi-squared=10.8, $p=0.001$) (Figure 1A).

Control for body-size has had mixed effects on study outcome: five studies found that significant HC abnormalities in ASD remained even when body size was included as a covariate(22,55,56,62,63), but five studies failed to find significant HC alterations in ASD after co-varying for body size(21,35,58,59,64).

Cross-sectional HC studies provide two types of summary metrics amenable to integration across multiple reports: macrocephaly rate and mean HC centile. These integrative analyses are fully detailed in the text and Figure S2 of Supplement 1 and Table S3 of Supplement 2. For the more commonly-used index of macrocephaly rate (Figure 1B), we found that (i) exclusion of the 38% of reports that estimated macrocephaly rates in ASD using HC norms that are now known to over-identify brain enlargement in contemporary samples of healthy children (Table 2) reduced the mean ASD macrocephaly rate across all studies from 14% to 4%, and (ii) studies that compared macrocephaly rates in ASD against those in contemporaneously ascertained controls were significantly less likely to identify significantly elevated macrocephaly in ASD when compared to studies comparing observed rates in ASD to the norm-predicted rate of 3% (Chi-squared=10.5, $p=0.002$). A parallel effect was seen for HC centile findings: similar group-mean HC centile estimates in ASD tend to have been differently interpreted based on whether expected (i.e 50th HC centile) or observed (i.e HC centile in recruited control sample) HC centile data were used as a comparison (see Figure S2 in Supplement 1).

Longitudinal Studies

Eleven (32%) of the 34 studies identified by our systematic review included a longitudinal comparison of HC in a group of participants with ASD to HC norms and/or controls (Figure 1C). The four norm-referenced longitudinal HC studies in ASD have all used CDC HC norms(15,16,19,20,67) and consistently found evidence for robust EBO in ASD during the first year of life. In contrast reports based on comparisons with locally-recruited controls have been mixed. The two control group referenced studies that impose linear HC trajectories within selected age-bands(22,24) replicate reports of EBO in ASD vs. CDC norms – i.e abnormally accelerated HC increase during the first year of life. Other control group referenced studies either find evidence of subtle EBO in ASD that emerges during the second year of life(17,18), or fail to find any evidence for abnormally accelerated HC growth in ASD(23,25).

Integrating Cross-Sectional and Longitudinal HC Data Between Birth and 18 Months

Of the 16 studies that provide HC data within the first two years of life, four provide sex-normed CDC HC centiles(15,19,20,35) and five others provide sex-specific estimates of mean raw HC that can be converted into HC centile(17,18,21,22,25). Taken together, these 9 studies provide information about HC growth in 470 children with ASD and 342 controls, using a total of 2005 and 1814 HC observations (respectively) (See Table S4 in Supplement 2). The deviant CDC HC centile trajectories that these studies all identify in ASD are mimicked by PCN norms - the largest and most recent description of HC growth in US children(30) (Figure 2A). This same pattern of HC overgrowth relative to CDC norms is also clearly evident in at least three(18,22,35) of the four(18,21,22,35) samples of typically developing children that have been included as controls in ASD studies (Figure 2B). Figure 2C shows cross-study average CDC centile trajectories of HC growth for clinically-ascertained children with ASD and control groups used in ASD studies, alongside the 50th centile for HC growth in PCN norms.

FINDINGS: ANALYSIS OF NEW HC DATA

Participant characteristics are summarized in Table 3.

Serial Cross Sectional Analyses

Distributions for raw HC did not show statistically significant violations from normality for either group at any age-point. There were no statistically significant group differences in raw HC at any age-point (Table 4). Head circumference centile distributions were significantly non-normal for both ASD and control groups at most age-points examined - although these deviations were more marked for CDC and WHO than PCN norms (see text and Figure S3 in Supplement 1 for more details). Nevertheless, to allow comparison with prior HC studies in ASD [which commonly report mean HC centile values at each age-point (Table S1 in Supplement 2)], we plot mean cross-sectional HC centile values for CDC, WHO and PCN in Figure 3. For HC in both the ASD and control group (i) mean CDC centile rises steeply from birth to reach a plateau at about 8 months, (ii) mean WHO centile is within expected limits at birth, but abnormally elevated thereafter, and (iii) mean PCN centile adheres most closely to the expected “flat” trajectory (Fig 3C).

Longitudinal Analyses

There were no statistically significant group differences in rate of HC change between any two HC measurement time points after correction for multiple comparisons (Table 4). Non-linear mixed modeling of HC growth (Figure 4A) did not identify any significant trajectory differences between ASD and control groups, although visual comparison of the best-fit trajectory for each group suggests a steady divergence of mean HC such that by 18 months, estimated mean HC in ASD is ~ 7mm greater than that in controls (equivalent to an effect size of 0.5*d* based on the pooled standard deviation of HC across ASD and control groups at age 18 months).

Our findings in cross-sectional and longitudinal HC analyses were robust to stratification by Developmental Regression Status and Control for Height and Demographic Variables (see text in Supplement 1).

Integration With Prior HC Data Between Birth and 18 Months

Figure 4B shows that HC growth for the newly-presented sample of children with ASD and controls replicates the pattern of HC overgrowth in the first year of life relative to CDC norms that has been found in previous studies of children with ASD and controls, as well as the largest and most-recently published set of US HC norms(30). This highly replicable pattern of EBO during the first year of life is abolished when HC values are re-expressed as their equivalent male PCN centile (Figure 4C): patterns of HC growth in previous studies and in our newly-presented cohort are broadly concordant with those predicted by PCN norms.

DISCUSSION

Our systematic review identifies several lines of evidence that the use of HC population norms has introduced systematic age-dependent biases in studies of early brain growth in ASD. We were able to replicate, and better specify the nature of these age-dependent HC norm biases by analyzing a new set of HC data in children with ASD against different sources of control data.

The Influence of HC Norm Use on Tests of the EBO Hypothesis

The vast majority (83%) of HC studies testing the EBO hypothesis in ASD involve the use of HC norms as comparison data. At first glance, these studies provide strong support for the hypothesis that children with ASD have an abnormal acceleration of brain growth in the first year of life(15,19,20,25,35), and that it persists as an HC excess once the early childhood years of rapid HC growth have passed(16,44,46,50,51,53,55,57). However, several findings in our study, when considered alongside existing knowledge regarding secular changes in HC growth [United Kingdom(28,36), China(37), Netherlands(38), Finland(32), Japan(39), Korea(40)] and the improper approximation of contemporary HC growth patterns by commonly used HC norms(28–33), suggest that the striking consistency of norm-based findings in ASD may reflect replicable biases in HC norms rather than a replicable pattern of dysregulated growth in ASD.

First, we show that comparisons of HC data between children with ASD and locally recruited controls are significantly less likely to identify HC abnormalities in ASD than are studies using HC norms. Second, removal of those cross-sectional reports that use HC norms with known biases towards the over-identification of HC enlargement, reduces the estimated mean macrocephaly rate in ASD from 14% to 4%. Third, and critical to the appraisal of evidence for EBO in ASD, we find that the reported developmental unfolding of EBO in ASD relative to CDC norms[(15,19,20,25,35) and the present study] - the most commonly used HC norm in ASD research – is closely mimicked by CDC HC centile growth in (i) control samples included in prior ASD studies (see Figure 2B and Figure S3 in Supplement 1), (ii) controls in our own study (see Fig 3), and (iii) ~500k HC observations in the most

recently published set of HC norms for US children [Figs 2 and 4 and(30)]. Thus, systematic review, and the new data analyzed here all find that HC growth in healthy children starts slightly below what is expected by CDC norms, crosses the 50th CDC centile at between 3 and 5 months of age, and continues to cross CDC centiles until stabilizing at approximately 8–10 months. These convergences indicates that prominent reports of such HC growth in children with ASD(15,19,20,25,35), which account for a large degree of the replicability across studies that test for EBO in the first two years of life, reflect replicable CDC norm biases rather than a disease-related phenomenon.

The evidence we present for norm-related biases has consequences that stretch far beyond research on aberrant early brain growth in neurodevelopmental psychiatry. Comparisons with population growth norms are an integral part of everyday clinical practice and are also frequently referred to in public-health settings(68). In both these contexts, decisions informed by growth norm use can carry significant cost and risk. Our study provides a strong and empirically-grounded argument for use of multiple growth norms in parallel, as well as comparison with data from typically developing and “clinical control” samples (e.g (23)) where possible.

Evidence for EBO in ASD from HC Studies that Minimize Norm-related Biases

Thirteen studies - six cross-sectional(56,62,63) and seven longitudinal(17,18,22–24)–have employed study designs that minimize the potential for norm-related biases when testing the EBO hypothesis. The bias-minimizing strategies are: (i) direct comparisons of raw HC in ASD to that in controls(17,18,21–25,56,60,62,63), (ii) epidemiological sampling of children with ASD and determination of HC centile relative to healthy controls identified within the same sampling frame(54), and (iii) selection of published HC norms that are secularly and demographically close to the ASD sample in question(23,58).

Of the six cross-sectional studies that minimize norm-related biases, three find evidence of HC enlargement in ASD(56,62,63), and three do not(54,58,60). The three negative reports all draw on population or broad screening-based samples of children with ASD. The three positive cross-sectional studies all examine clinically ascertained samples and identify mild (4–7mm) degrees of HC enlargement among males with ASD [two after stratification by sex(62,63) and one by virtue of only including males(56)]. The reported effect size for these HC excess in ASD is $\sim 0.5d$, which converges with that found in our sample, and is approximately half that identified in reports of mean HC excess in ASD relative to controls (see Fig 2).

Of the six longitudinal studies that minimize norm-related biases, four(17,18,22,24) identify periods in early childhood when the rate of HC increase in clinically ascertained children with ASD is faster than that in controls. Two(22,24) of these four positive studies force HC growth to be linear between selected “breakpoints”, and identify an early phase of abnormally accelerated HC growth in ASD which coincides with that arising through bias in CDC norms(15,19,20,25,35). In contrast, the two positive longitudinal studies that model HC growth using more biologically valid exponential growth curves find that the group-average ASD growth curves are essentially identical to those in controls until the second year of life(17,18). These four positive studies stand alongside three negative longitudinal

reports – one of which finds that growing differences between HC in ASD and typically developing controls are in keeping with more generalized growth abnormalities(21), and another two which find no evidence of accelerated HC growth in children with ASD relative to controls(23,25).

Thus, to the extent that existing (albeit non-epidemiological) data support the presence of a generalized tendency towards EBO in ASD, this overgrowth appears to be much more subtle and later-onset in nature than picture of gross overgrowth during infancy that emerges due to norm-biases. These distinctions are biologically relevant given that regional contributions to global brain volume change vary so markedly between the first and second year of life (69).

Summary and Next Steps

In conclusion, the most methodologically robust and bias-free sources of evidence regarding HC growth in ASD – longitudinal studies that model and compare HC growth curves in ASD to those in carefully matched controls(17,18,21,23,25) – are equivocal regarding the presence of abnormally accelerated early brain growth in ASD. Where evidence for EBO has been found, HC growth in ASD begins to show a subtle divergence from that in controls during the second year of life(17,18). This pattern of EBO is distinct from the dramatic brain overgrowth before age 1 year that is suggested by CDC-normed analyses(15,19,20,25,35), and its presence has yet to be established in population-based samples of children with ASD. This latter limitation is of particular concern given that well-established demographic differences between children with ASD who are known to clinical services vs. those that remain undiagnosed[such as maternal educational level(70)], are also highly correlated with HC growth in early childhood(71). Such confounds mean that recruitment biases in non-epidemiological clinical samples may lead to artifactual case-control differences in HC growth. With appropriate consideration of these methodological limitations, studies of HC may still provide a valuable tool for biological research in ASD however by virtue of being much more amenable than brain structural MRI to longitudinal research in large population-based samples.

Moving forward, it will be critical to determine if epidemiologically ascertained samples of children with ASD show longitudinal disruptions of HC growth relative to norms defined within the same population-based reference frame or contemporaneous local controls (rather than historical norms). Analyses of HC growth in ASD within more recently established population-based cohorts(72) could address these limitations however, and also help to clarify if aberrant brain growth in ASD segregates with characteristics such as sex and regression status(22).

While a generalized tendency towards HC increase in ASD remains to be established, there is no doubt that brain enlargement is a consistent feature of several genetic syndromes that are accompanied by an ASD-like behavioral phenotype(73). Of note, however, the opposite is also true as genetic studies have also shown that ASD-like behaviors are a clinical feature of several microcephalic syndromes(74). One hypothesis therefore is that extreme dysregulation of brain growth may be more relevant to our understanding of ASD than brain enlargement *per se*. Unlike existent applications of HC norms to clinically ascertained samples of children with ASD (which have largely failed to find significantly elevated

microcephaly rates), forthcoming population-based studies with rich genomic and environmental information(75) should be able to compare the mutually exclusive “extreme-growth” vs. “isolated over-growth” hypotheses, and link instances of aberrant brain size to candidate genetic/environmental influences during prenatal or early postnatal life.

Beyond clarifying the presence and nature of EBO in ASD, and the extent of any accompanying association between ASD and microcephalic syndromes, future research will ultimately need to determine where disruptions of overall brain growth in ASD sit on the spectrum between epiphenomenal biomarker to causal intermediate phenotype (76). The answer to this question - which will rely heavily on longitudinal and genetically informative studies in developing humans [e.g IBIS (Infant Brain Imaging Study)(77)] – ultimately determines the potential for continued study of EBO to improve etiological understanding and clinical management of ASD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thanks the children and families who took part in this study. Financial support for this work was provided by the NIH Intramural Research Program.

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A: CROSS-SECTIONAL HEAD CIRCUMFERENCE STUDIES

B: MACROCEPHALY REPORTS

C: LONGITUDINAL STUDIES

FIGURE 1. Systematic Review Findings

1A) Cross-sectional studies (in reverse order of publication year) grouped according to use of HC norms vs. locally recruited controls as a comparison for ASD data: This grouping was significantly associated with study outcome (Chi-squared=7.5, $p=0.006$). A “negative” study outcome was also associated with a narrower study age-range ($t=2.3$, $p=.03$). Each study is a horizontal bar spanning the study age-range, with study outcome coded by color, and density of HC observations coded by bar height. Where one study reported multiple cross-sectional tests the coded outcome relates to analysis of raw HC where available and HC centile where not. Outcomes are coded before any control for body size. **1B)** Summary of reported macrocephaly rates in ASD grouped according to use of expected macrocephaly rate in HC norms (3%) or observed macrocephaly rate in controls as a comparison: Each reported rate

is a point, with significance of test for macrocephaly enrichment in ASD coded by shape, HC norm is coded by color, and density of HC observations coded by size. Study stratification by comparison group was significantly associated with study outcome (Chi-squared=10.5, $p=0.005$). **1C)** Longitudinal studies grouped according to use of HC norms vs. locally recruited controls as a comparison for ASD data: Each study is a horizontal bar spanning the study age-range, with periods of abnormal HC change in ASD coded by color, and density of HC observations coded by bar height.

FIGURE 2. Reported Patterns of HC Growth Between Birth and 18 Months within the Common Framework of CDC Head Circumference Reference Norms

The trajectory for expected growth according to CDC norms (50th centile) is shown as a horizontal solid black line. The trajectories for expected growth according to PCN norms (50th centile) is shown as dashed black line. **2A)** Prior reports in ASD: Each study is a differently colored solid line. **2B)** Prior reports for control groups include in studies of HC in ASD: Each study is a differently colored solid line. **2C)** Summary of weighted mean ASD (solid red) and control (solid blue) HC trajectories. Confidence intervals are not shown as constituent studies did not uniformly provide estimates of error for reported group HC means.

FIGURE 3. Estimates of Mean HC centile (with 95% Confidence Intervals) for ASD and Control Participants at Each Age-point According Different HC Norms

A) CDC, B) WHO and C) PCN HC norms. For HC in both the ASD and control group (i) mean CDC centile rises steeply from birth to reach a plateau at about 8 months, (ii) mean WHO centile is within expected limits at birth, but abnormally elevated thereafter, and (iii) mean PCN centile adheres most closely to the expected “flat” trajectory.

FIGURE 4. Growth Curve Modeling of HC Data in New Cohort and Combination of New Cohort Findings With Summaries From Systematic Review

A) Patterns of HC change in new cohort of participants with ASD and controls. Each person is depicted as a set of connected points, with overall group-best fit trajectories shown as bold solid lines: Color-coded for group (ASD-red, controls-blue). B) Group best-fit longitudinal HC trajectories in new cohort and weighted mean HC trajectories generated by systematic review, shown as CDC centile. The trajectories for expected growth (50th centile) according to CDC norms is shown as a horizontal solid black line. The trajectories for expected growth (50th centile) according to PCN norms are shown as dashed black line. C) Group best-fit longitudinal HC trajectories in new cohort and weighted mean HC trajectories generated by systematic review, shown as PCN centile. The trajectories for expected growth (50th centile) according to PCN norms is shown as a horizontal solid black line. Confidence intervals are not shown as constituent studies did not uniformly provide estimates of error for reported group HC means.

TABLE 1

Summary of Studies Included in Systematic Review.

FIRST AUTHOR	YEAR	ANALYTIC APPROACH	CASES	COMPARISON DATA			HC SUMMARY USED	CONTROL FOR HC MODIFIERS			FINDINGS: CODED				
				DIAGNOSIS	SOURCE	SAMPLE SIZE		AGE RANGE (months)	HC observations per months	HC norm < 3 years	HC norm > 3 years	CONTROLS	SEX	RACE	BODY SIZE
Bolton	1994	C	27	60–192	0.2	-	TANNER	N	Centile Cut Offs	Y	Y	N	↑	-	-
Davidovich	1996	C	148	8–84	2	NELLHAUS	NELLHAUS	N	Centile Cut Offs	Y	N	N	↑	-	-
Woodhouse	1996	C	37	60–192	0.2	-	TANNER	N	Centile Cut Offs	Y	Y	N	↑	-	-
Lainhart	1997	C	91	0–456	0.2	ROCHE	ROCHE	N	Mean and Centile Cut-Offs	Y	N	N	↑	-	-
Rodier	1997	C	61	72–168	0.7	-	FEINGOLD	Y	Centile Cut Offs	Y	Y	N	→	-	-
Fombonne	1999	C	126	14–192	0.7	SEMPE	SEMPE	N	Centile Cut Offs	Y	N	N	↑	-	-
Fidler ^a	2000	C	41	60–192	0.3	ROCHE	ROCHE	N	Centile Cut Offs	Y	Y	N	↑	-	-
Miles	2000	C	137	12–492	0.3	NELLHAUS	NELLHAUS	N	Mean and Centile Cut Offs	Y	N	N	↑	-	-
Gillberg ^b	2002	C	100	0–192	44	KARLBERG	KNUDTZON	N	Mean	Y	N	N	↑	-	-
Courchesne ^c	2003	C and L	48	0–14	7.5	CDC	-	N	Mean	Y	N	N	↑	↑	-
Deutsch	2003	C	63	52–168	0.5	-	FARKAS	N	Mean and Centile Cut-Offs	Y	Y	N	↑	-	-
Torrey	2004	C	15	0–84	3.5	-	-	Y	Mean and Centile Cut-Offs	Y	Y	N	→	-	-
Dementieva	2005	C and L	251	0–216	42	CDC	ROCHE	N	Mean and Centile Cut-Offs	Y	N	N	↑	↑	-
Hazlett	2005	C and L	113	0–48	2.7	CDC	-	Y	Mean	Y	Y	Y	→	↑	↑
Dissanayake	2006	C and L	28	0–36	0.8	-	-	Y	Mean	Y	N	N	→	↑	-
Lainhart ^d	2006	C	208	36–564	21.7	ROCHE	ROCHE	Y	Mean and Centile Cut-Offs	Y	Y	Y	↑	-	↑
Dawson	2007	L	28	0–36	5	CDC	-	N	Mean	Y	N	Y	↑	↑	-
Mills	2007	C	71	48–96	1.5	CDC	-	Y	Mean	Y	Y	Y	↑	-	↑
Mraz ^e	2007	C	35	0–24	1.5	CDC	-	Y	Mean	Y	Y	Y	→	-	→
Sacco	2007	C	241	36–192	1.5	NCHS	BUSHBY	N	Centile Cut-Offs	Y	N	N	↑	-	-
van Daalen	2007	C	53	1–14	53	FREDRIKS	-	Y	Mean	Y	Y	Y	→	-	→
Webb	2007	C and L	28	0–36	8.3	CDC	-	N	Mean	Y	Y	N	↑	↑	-
Fukunoto	2008	C	85	0–12	42	JAPANESE NORMS	JAPANESE NORMS	N	Mean	Y	Y	Y	↑	-	→
Constantino	2010	L	48	0–36	7.7	-	-	Y	Mean	Y	N	N	-	↑	-

FIRST AUTHOR	YEAR	ANALYTIC APPROACH	CASES			COMPARISON DATA			HC SUMMARY USED	CONTROL FOR HC MODIFIERS			FINDINGS: CODED				
			DIAGNOSIS	SOURCE	SAMPLE SIZE	AGE RANGE (months)	HC observations per months	HC norm < 3 years		HC norm > 3 years	CONTROLS	SEX	RACE	BODY SIZE	HC	HC CHANGE	CONTROLLING FOR BODY SIZE
Barnard-Brak	2011	C	ASD	EPIDEMIOLOGIC	100	9–36	100	WHO	-	Y	Mean and Centile Cut-Offs	Y	N	N	→	-	-
Chawarska ^f	2011	C and L	ASD	CLINIC	98	0–24	98	CDC	-	Y	Mean and Centile Cut-Offs	Y	Y	Y	↑	↑	→
Davidovitch	2011	C	ASD	CLINIC	317	8–84	4	CDC	NELLHAUS	Y	Mean and Centile Cut-Offs	Y	N	N	↑	-	-
Fukumoto	2011	C	ASD	CLINIC	280	0–12	136	-	-	Y	Mean	Y	Y	Y	↑	-	↑
Nordahl	2011	C and L	ASD	CLINIC	78	0–18	26	-	-	Y	Mean	Y	Y	Y	↑	↑	↑
Ozgen	2011	C	ASD	CLINIC	224	36–216	1.2	-	-	Y	Mean	Y	Y	Y	↑	-	↑
Whitehouse	2011	C	ASD	COMMUNITY	14	0	14	BEEBY	-	Y	Mean	Y	Y	Y	→	-	→
Rommelse	2011	C and L	ASD	COMMUNITY	129	0–19	6.8	FREDRIKS	-	Y	Mean and Centile Cut-Offs	Y	N	Y	→	-	↑
Gray	2012	C and L	AUT	CLINIC	75	0–69	37	CDC	-	Y	Mean	Y	N	N	↑	→	-
Ververi	2012	C	ASD	CLINIC	222	18–108	2.5	CHIOTIS	CHIOTIS	N	Centile Cut+Offs	Y	N	N	↑	→	-
Raznahan	2012	C and L	AUT	COMMUNITY	37	0–18	27	CDC, PCN, WHO	-	Y	Mean and Centile Cut-Offs	Y	Y	Y	→	→	→

See Table S1 for more detailed study information, and see Table S2 for rules applied during data abstraction (both in Supplement 2).

Abbreviations: - (not applicable), C (Cross-sectional), L (Longitudinal), ASD (Autism Spectrum Disorder), Y (Yes), N (No), CDC (Center for Disease Control), PCN (Primary Care Network), WHO (World Health Organization), ↑ [Evidence for abnormal brain enlargement (if cross-sectional finding), or accelerated brain growth (if longitudinal finding)], → (No evidence found for brain enlargement or accelerated brain growth).

Notes:

^a information about age range was not available from paper or authors, and is therefore taken to be 5–16 yrs for calculation of HC observations per month,

^b reported macrocephaly rates not abstracted as only provided relative to body size,

^c longitudinal analyses compared ASD participants to a sub-set individuals included in Roche HC norms,

^d required information (head size data for those under 18 years of age) only available for the Autism sub-sample of a larger (n=338) ASD cohort,

^e classified as a negative cross-sectional study since no evidence of brain enlargement relative to controls,

^f classified as a longitudinal study based on use of repeat measures to model trajectory of HC growth, but no direct test made for differences in rate of HC change between ASD and controls.

For the 34 studies identified, more recent studies have tended to have a “lighter” age-range (p=0.04) and greater density of HC observations (p=0.02) [As determined by linear regression of publication year on “age range” and “HC observations per month” respectively]. The number of HC studies published per year has risen steeply in the past 10 years, with recent studies more often using longitudinal as opposed to purely cross-sectional designs. With the exception of two epidemiologically based studies(48,60), and three that identified clinical participants through broad-based screening efforts(23,54,78), all available studies examine clinically-ascertained samples of children with ASD. In terms of clinical profile, most studies [excepting (18,63)] have examined groups of participants with ASD unselected for general cognitive ability. While most studies have sought to exclude cases of ASD associated with potential causal genetic syndromes, the comprehensiveness of laboratory-based assays for such syndromes has been highly variable. Control for known modifiers of HC has been mixed: while all studies have accounted for sex differences in HC by use of sex-specific norms or stratification by sex, control for potential race and body size influences on HC has been much less consistent (<30% of studies simultaneously control for race and body size effects on HC).

TABLE 2
Head Circumference Reference Norms Used to Test the EBO Hypothesis in ASD

Details for HC norms used in HC tests of the EBO hypothesis. Three norm sources are not included as the required information could not be obtained (Chiotis et al., 2003; Education, 2001; Feingold and Bossert, 1974).

HC NORM CHART	AGE-RANGE (YEAR OF COLLECTION)	SOURCE / ETHNICITY	REPRESENTATIVE HC CENTILES	AGE 1 YEAR			USES IN ASD RESEARCH (c.f. Table 1)	RECENT EVIDENCE FOR BIAS TOWARDS OVERESTIMATING HC CENTILE
				AGE 10 YEARS				
				50th	97th	97th		
TANNER (1966)	0–19yrs (1966)	UK / Caucasian	46.7	49.6	52.7	55.2	Bolton (1994), Woodhouse (1996)	Wright(2002) Ounsted(1985)
NELHAUS (1968)	0–18yrs (1943 – 1965)	US, MULTICENTER EUROPE / Caucasian and African-American	47	49.6	53	55.9	Davidovitch(1996, 2011), Miles (2000)	No studies available
NCHS (1979)	0–3yrs (1930 – 1975) “Fels data” 2–18yrs (1962 – 1974)	USA / Caucasian USA / Caucasian	47	-	-	-	Sacco (2007)	No studies available
SEMPE (1979)	0–19 yrs (1953–1954)	FRANCE / Caucasian	47	49.4	53.1	55.7	Fombonne (1999)	No studies available
ROCHE (1987)	0–18yrs (1930 – 1982) “Fels data”	USA/Caucasian	47	49.6	53.5	56.5	Lainhart(1997, 2006), Fidler (2000), Dementieva (2005),	No studies available
KNUDTZON(1988)	0–4 (1982–1984) 3–17 (1971–1974)	NORWAY/Caucasian	46.5	49.2	53.9	55.7	Gillber (2002)	No studies available
FARKAS (1994)	6–18yrs (1973–1976)	CANADA / Caucasian	-	-	53.5	56.3	Deutsch (2003)	No studies available
CDC (2000)	0 (1960–1994) “Fels data” 2mths – 3yrs (1971–74 “NHANES I”) 6mths – 3yrs (1976–80 “NHANES II”) 2mths – 3yrs (1988–94 “NHANES III”)	USA / Caucasian USA / “Nationally Representative” USA / “Nationally Representative” USA / “Nationally Representative”	46.2	48.7	-	-	Courchesne (2003), Dementieva (2005), Hazlett (2005), Dawson (2007), Mills (2007), Mraz (2007), Webb (2007), Chawarska (2011), Davidovitch (2011), Gray (2011)	Karvonen(2012) Daymont(2010). Schienkewitz(2011) Werner(2006)
FREDRIKS (2000)	2wks – 21yrs (1996–1997)	THE NETHERLANDS / Caucasian	47.2	50.2	53.3	56.7	van Daalen (2007), Rommelse (2011)	No studies available
WHO (2006)	0–5yrs (1997–2003)	GLOBAL / Multiethnic	46.1	48.5	-	-	Barnard-Brak (2011)	Daymont(2010) Juliusson(2011) Schienkewitz(2011)

Abbreviations: NCHS (National Childrens Health Study), CDC (Center for Disease Control), WHO (World Health Organization)

TABLE 3

Participant Characteristics.

Characteristic	CONTROL	ASD	Group Difference
Number	22	35	
ADOS severity score, mean (s.d)	1 (0.4)	7 (4.1)	p<0.0005
Full Developmental Quotient (DQ), mean (s.d)	113 (11.3)	53 (18.4)	p<0.0005
Verbal DQ, mean (s.d)	111 (17.9)	45 (22)	p<0.0005
Non-verbal DQ, mean (s.d)	115 (12.3)	61 (17.2)	p<0.0005
SES, mean (s.d)	59 (3.9)	56 (7.1)	p=0.07
Age at HC Measurement (months), mean (s.d)			
Birth (N CONTROLS = 18/ N ASD= 26)	0.01 (0.02)	0.04 (0.07)	n.s
2 month age-point (18/27)	1.95 (0.15)	2.04 (0.16)	n.s
4 month age-point (16/27)	4.04 (0.1)	4.03 (0.13)	n.s
6 month age-point (18/24)	6.03 (0.16)	6.07 (0.16)	n.s
9 month age-point (17/25)	9.01 (0.13)	9.07 (0.14)	n.s
12 month age-point (13/22)	12.08 (0.09)	12.15 (0.15)	n.s
15 month age-point (16/26)	15.08 (0.52)	15.23 (0.35)	n.s
18 month age-point (13/24)	18.20 (0.53)	18.34 (0.42)	n.s
Total Number of HC Measures	129	201	
Mean Number of HC Measures Per Person	5.9	5.7	

Abbreviations: s.d (standard deviation), HC (Head Circumference), SES [Socioeconomic Status as estimated by (Hollingshead, 1975)].

Serial Analysis of Head Circumference at Each Age-point and HC Change Between Each Pair of Age-points
TABLE 4

The borderline-significant $p=0.05$ 6mm excess in mean ASD HC relative to controls at age 6 months did not survive correction for multiple comparisons (corrected p value=0.4). Group differences in raw HC change approached statistical significance for the interval between 2 and 4 months of age when the HC increase in controls was greater than that for children with ASD ($p=0.01$). This isolated group difference did not survive correction for multiple comparisons (corrected p value=0.006). For the sub-set of participants with complete HC data at birth and 18 months (12 CONTROLS and 19 ASD), total HC change between birth and 18 months was normally distributed and statistically indistinguishable between groups (Mean(s.d) HC increase in cm: CONTROLS – 14.2(1.1), ASD – 14.3(1.4), t -test for difference $p=0.83$).

AGE (months)	HEAD CIRCUMFERENCE			AGE DIFF		HEAD CIRCUMFERENCE CHANGE			
	CONTROLS		ASD	n	p	CONTROLS		ASD	
	n	mean(sd)	n mean(sd)			n	median(range)	n	median(range)
0	18	34.6(1.23)	26 34.4 (1.26)	0.2	-	-	-	-	-
2	18	39.4(1.31)	27 39.8(2.10)	0.4	0-2	15	5 (3-7)	20	6 (2-8)
4	16	42.4(1.0)	27 42.5(1.24)	0.7	2-4	16	3 (2-5)	25	2 (1-5)
6	18	44.1(1.04)	24 44.7 (1.53)	0.05	4-6	15	2 (0-3)	24	2 (0-4)
9	17	45.9(1.20)	25 46.1(1.47)	0.5	6-9	16	2 (1-3)	21	2 (0-3)
12	13	46.7(1.01)	22 47.4(1.44)	0.1	9-12	12	1 (0-2)	18	1 (1-3)
15	16	47.7(1.04)	26 48.2(1.6)	0.2	12-15	11	1 (0-1)	20	0.5 (0-2)
18	13	48.5(0.9)	24 49.1(1.5)	0.2	15-18	11	1 (0-2)	21	1 (0-2)