Title

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Journal

Biological psychiatry, 74(8)

ISSN

0006-3223

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Publication Date

2013-10-01

DOI

10.1016/j.biopsych.2013.04.018

Peer reviewed

Published in final edited form as:
Biol Psychiatry. 2013 October 15; 74(8): 576–584. doi:10.1016/j.biopsych.2013.04.018.

Adjusting head circumference for covariates in autism: clinical correlates of a highly heritable continuous trait

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Financial Disclosures Dr. Cathy Lord receives royalties for the scales of diagnostic instruments for which she's a coauthor; she donates all royalties to autism charities. All other authors reported no biomedical financial interests or potential conflicts of interest.

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Abstract

BACKGROUND—Brain development follows a different trajectory in children with Autism Spectrum Disorders (ASD) than in typically developing children. A proxy for neurodevelopment could be head circumference (HC), but studies assessing HC and its clinical correlates in ASD have been inconsistent. This study investigates HC and clinical correlates in the Simons Simplex Collection cohort.

METHODS—We used a mixed linear model to estimate effects of covariates and the deviation from the expected HC given parental HC (genetic deviation). After excluding individuals with incomplete data, 7225 individuals in 1891 families remained for analysis. We examined the relationship between HC/genetic deviation of HC and clinical parameters.

RESULTS—Gender, age, height, weight, genetic ancestry and ASD status were significant predictors of HC (estimate of the ASD effect=0.2cm). HC was approximately normally distributed in probands and unaffected relatives, with only a few outliers. Genetic deviation of HC was also normally distributed, consistent with a random sampling of parental genes. Whereas larger HC than expected was associated with ASD symptom severity and regression, IQ decreased with the *absolute value* of the genetic deviation of HC.

CONCLUSIONS—Measured against expected values derived from covariates of ASD subjects, statistical outliers for HC were uncommon. HC is a strongly heritable trait and population norms for HC would be far more accurate if covariates including genetic ancestry, height and age were taken into account. The association of diminishing IQ with absolute deviation from predicted HC values suggests HC could reflect subtle underlying brain development and warrants further investigation.

Keywords

head circumference; body metrics; genetic ancestry; IQ; autism spectrum disorder; ASD

Autism spectrum disorders (ASDs) are a group of heterogeneous neurodevelopmental disorders causing significant social, communication, and behavioral deficits and challenges (1). Increased head circumference (HC) is one of the most replicated clinical findings in

autism and there is now ample evidence of accelerated brain growth in early childhood (2–5).

HC studies typically report increased rates (11~27%) of macrocephaly (HC≥97th percentile or 2 standard deviations) in ASD compared with general population (6–16). However, some studies suggested that the use of national normative samples as control data could introduce biases including ancestry and secular effect (17–19). The role of height as another confounding variable remains elusive. Normally, HC correlates closely with height (20,21). However, results have been conflicting, with several studies suggesting that height should be considered as a covariate (18,22,23), whereas others suggest that increased HC is independent from an increase of height (12,24). Additionally, a pilot study suggested that abnormal growth may be related to psychiatric disorders in general (25).

Whether macrocephaly identifies a neurobiological subtype of autism is not clear yet. Some authors suggest that macrocephaly is an endophenotype pointing towards specific etiopathogenic factors (22), whereas others consider it as the tip of the iceberg of a more general tendency toward increased HC (11,24). Two studies have commented on a more general overgrowth syndrome (22,23). Additionally, studies evaluating clinical factors associated with macrocephaly find somewhat diverse results. Indeed, reports of clinical correlates of HC in autism, including adaptive function (10), autism symptom severity (23), language development (22,24), regression (26), as well as IQ scores (22), have been inconsistent, most likely because of insufficient adjustment for covariates and lack of precision due to small sample sizes.

This study aimed to investigate clinical correlates of HC in a large sample, after carefully adjusting for covariates, to clarify the role of potential confounds. For this, we conducted a multi-step analysis of 2644 Simons Simplex Collection (SSC) families, using a mixed model that provided (i) estimates of covariates' effects and (ii) estimates of the deviation from the expected HC given parental HC. This model not only allowed us to determine the role of several covariates on HC to accurately test if ASD status was a predictor of HC, but also to examine the relationship between HC and previously reported clinical correlates of HC in autism.

METHODS

Sample

SSC is a cohort of simplex families of children with ASD. Data on 2644 families, 2185 quad (a proband with an ASD, both biological unaffected parents, and one or more unaffected siblings), and 459 trio (no siblings), were extracted from the SSC database, v14.1 (http://sfari.org/resources/sfari-base). All individuals were older than 4 years. Instruments used to assess the phenotypes are available on the Simons Foundation Autism Research Initiative website (https://sfari.org/). Please see the Supplement for inclusion/exclusion criteria.

Each SSC center designated one person to measure all participants HC (for protocol, see Supplement), height and weight. Also provided were self-reported ancestry, ASD status, sex, and age at measurement in months. To examine the relationship between HC and clinical parameters, we used data available for the proband on the Autism Diagnostic Interview-WPS edition (ADI-WPS)(27), Autism Diagnostic Observation Schedule-WPS edition (ADOS-WPS)(28), Vineland Adaptive Behavior Scale-2nd edition (VABS-II)(29). Intellectual quotient (IQ) was evaluated either with the Differential Ability Scales-2nd edition (DAS-II)(30), Wechsler Intelligence Scale for Children-4th edition (WISC-IV)(31), Mullen Scales of Early Learning(32), or the Raven's standard progressive matrices(33). When children had the Raven's, verbal IQ was estimated from the Peabody Picture

Vocabulary Test-4th edition (PPVT-4)(34). SSC families were genotyped on the Illumina Infinium® 1Mv3 (duo) or the Illumina Infinium® 1Mv1 microarrays, providing genome wide genotype data to estimate genetic ancestry. After editing the data for missing information, a total of 1891 families remained for analyses, including 1891 fathers, 1891 mothers, 1889 probands (4 parents of two probands with incomplete data were included because they were informative for parental data analyses), and 1554 siblings (Table S1 in Supplement).

Models

Data were analyzed using two distinct mixed models fitted with QXPAK v.5.05 (36,37), a software package specifically developed to provide mixed model solutions to genomic data (http://www.icrea.cat/Web/OtherSectionViewer.aspx?key=485&titol=Software:Qxpak).

A single trait model analyzed the body metrics one at a time, with predictors including sex, ASD status, age deviation as a linear and quadratic effect component, and genetic ancestry. The model also included a random genetic effect for the individual. We used the family structure to account for genetic correlations among family members. Age deviation was set to 0 for individuals 21 years or older while the value of this covariate was determined as (252-age)/12 for all others, with age recorded in months. As an alternative to self-reported ancestry, we estimated genetic ancestry of all parents with whole genome high quality SNP genotypes (5156 SNPs with MAF>0.01, NCR<0.002), using GemTools (35). GemTools identified seven significant ancestry eigenvectors (EVs) from parents. Ancestry eigenvectors for the children were subsequently determined as the average of the two parental eigenvectors sets. Eigenvectors were adjusted such that the average of the adult European (self-reported ancestry) males equaled to 0. Self reported ancestry showed reasonable agreement with genetically inferred ancestry (Supplement: Table S4 and Figure S1). EV1 separated the European from the African and Asian ancestry; EV2 delineated African-Americans from Asians, EV3 showed a cline in the Europeans; EV5 separated two Asians groups, likely Chinese and Indian ancestry. EV4 separated Asians and Latinos. The model for HC included height and weight as covariates. We fitted these terms as deviations from the average adult male of European self-reported ancestry, height deviate=height-179.5 and weight deviate=weight-95.5. All covariates other than sex were nested within sex. In addition to estimating covariates, this model yielded a heritability estimate for HC.

In a multiple trait model, height, weight and HC were fitted simultaneously. All covariates of the single trait model were included. In addition to estimating effects of covariates and heritabilities, this model estimates phenotypic and genetic correlations among body metrics.

Residual HC and genetic deviation of HC

After fitting models, residual HC (residuals) was calculated as deviation of the observed HC from its expectation based on the effects of covariates. The mixed model also supplied estimates for the genetic contribution (GC) of each individual. Assuming that many genetic variants affect the observation (i.e., the infinitesimal model) and Mendelian inheritance, the GC of an individual (GC_i) is the sum of the average of the GC of the two parents (GC_f, GC_m) and a term accounting for random sampling of parental genetic variants (similar to an error term in a standard linear model). In expectation, children achieve the average of parental genes. However, each child gets a random sample of parental variation, causing deviation from the expected phenotype. Henceforth, we will use “genetic deviation of HC (genetic deviation)” for this deviation, which is computed from the genetic contribution of the child and the parents: genetic deviation=GC_i-(GC_f+GC_m)/2. Differences in distribution of genetic deviation in probands and sibs were tested using t-tests and the Kolmogorov-Smirnov (KS) test.

Analysis of outliers

Because a portion of this analysis involved normative data established in a sample of European ancestry, we targeted individuals with European self-reported ancestry. Analysis was run in 1458 probands, 1211 siblings, 1556 fathers and 1532 mothers. Raw HC data were converted to standardized z-scores using reference data (38). Because there were no HC normative data available for adults, parents' values were estimated by extrapolation from normative scores at 18 years of age. Individuals were “clinical outliers” if |z-score|>2). Outliers from the model were defined in two different ways: individuals with |genetic deviation|>2SD, with the SD computed from the variance in unaffected siblings (only siblings labeled S1, n=1146 siblings); from the normal mixture modeling using (http://cran.r-project.org/web/packages/outliers/index.html) to define statistical outliers. This mixture model analysis was also performed using all probands.

Relationship between HC and clinical parameters in probands

For HC, both |residuals| and |genetic deviation| were used as predictors in separate models. In addition to their absolute values, the difference between each HC variable and its mean was evaluated as a predictor. First the relationship between HC and IQ scores (as verbal, nonverbal and full IQ scores) was fitted, using linear models and smooth splines Then the relationship between HC and clinical factors was evaluated: adaptive behavior (as composite score of VABS-II), onset of first words and onset of first phrase (as items 9 and 10 of ADI-WPS), and autism symptom severity (as calibrated severity score of ADOS-WPS)(39) using linear regression models with full scale IQ as a covariate. The relationship between HC and history of regression (as appeared in the SSC database) was also estimated using a logistic regression model. All clinical parameters models were fitted with and without sex as covariate, and with and without interaction between sex and HC to identify a differential effect of HC between sexes on these parameters.

RESULTS

Heritability of body measures

The single and multiple trait heritability estimates for HC were very similar, 0.63 (95% CI 0.58–0.68) vs 0.65 (95% CI 0.62–0.68). The multiple trait model estimated heritability of 0.52 (95% CI 0.49–0.55) and 0.29 (95% CI 0.26–0.32) for height and weight, respectively, with phenotypic correlations among the traits of approximately 0.33 for all three combinations (Supplement: Table S3). Estimating heritability of HC separately for the 1533 probands from quads and 356 from trios produced similar estimates, 0.62 versus 0.64, which were not significantly different (p=0.88).

Several significant predictors of HC including ASD status

In the single trait model, ASD status was a significant predictor of HC in both males (p=0.00095) and females (p=0.0332) (Table 1). Probands had slightly larger heads, on average, compared to siblings, with a difference of approximately 0.2cm in both sexes. The R^2 for models with and without ASD status was 68.17% and 68.12% respectively, indicating that the predictive value of ASD status is low. Both height and weight were significantly correlated with HC, with taller and heavier individuals tending to have larger HC. The pattern was the same for both sexes and the effects were of similar magnitude, changing 0.05cm per 1cm in height and 0.035cm for every 1kg in weight. For both sexes, age, EV2 and EV5 were significant (Table 1 and Supplement: Table S2), whereas EV1 was significant only for data from females. A good model for expected HC as a function of age, sex, and self-reported ancestry was obtained (Figure 1), and plotting residuals as a function of age showed no pattern (Supplement: Figure S2), suggesting age is modeled appropriately. Type

of family (quad or trio) was not a significant predictor (p=0.59); thus trios and quads were analyzed together in all models.

Height and weight in probands versus siblings

Despite the differences between the multiple trait versus single trait approach to modeling the influence of height and weight on HC, the general trend of important covariates in predicting HC stayed the same (Tables 1 and 2). When modeling the three body metrics simultaneously in the multiple trait model, the effect of ASD status on HC remained significant. Probands were slightly shorter and heavier than their siblings, but these differences were not significant (Table 2). Thus the larger HC of probands, versus their unaffected siblings, was more likely to arise due to differential brain growth than to a generalized overgrowth phenomenon.

Distribution of residuals and genetic deviation in probands and siblings

After fitting the main effects of the model, including ASD status, the distributions of the residuals were symmetric for both probands and siblings (Supplement: Figure S3) and not significantly different (KS test p=0.24). Moreover, after fitting the genetic model, the distributions of genetic deviation for probands and siblings were not significantly different (KS test p=0.53), consistent with random sampling of parental genetic variants.

Only few true statistical outliers observed in probands

Applying established method used in pediatric clinics, i.e., z-scores derived from normative data (38), there were high rates of macrocephaly (z-score>2.0) not only in probands (14.7%), but also in unaffected relatives:12.7% of fathers; 19.6% of mothers and 12.9% of siblings. Very few of the probands (1.2%) or unaffected relatives (0.9% of the mothers, 0.5% of the fathers and 0.8% of the siblings) had microcephaly (z-score<−2.0).

Adjusting for covariates and parental HC, however, created a different picture. When defining outliers as |genetic deviation|>2 SD (SD=0.53), only 53 out of 1458 probands (3.6%) were outliers with large HC, 36 out of 1458 (2.4%) were outliers with small HC. The results were similar for siblings: (1.8% and 1.3% respectively). Moreover, fitting a mixture model, 36 probands (2.4%, 18 with a smaller and 18 with a larger HC than expected) and no sibling were classified as outliers with a probability>0.5, (Supplement: Figure S4). Thus, most of the individuals with z-score>2 were not statistical outliers (Supplement: Figure S5). When extending the clustering analysis to the whole sample of probands, regardless of ancestry, only 50 statistical outliers were found.

HC and clinical characteristics

Sex was significantly associated with IQ measures and it did not interact with HC for any model. Full scale IQ had a significant effect on the other clinical characteristics. Thus, sex was included in the model predicting IQ and IQ was included in the other models.

There was a negative linear relationship between the absolute value of HC and IQ scores: individuals with increasingly extreme HC showed increasingly lower mean IQ scores (Table 3, Figure 2 and Supplement: Figure S6). The predictive value of the models, as measured by partial R^2, was small. In contrast, there was a significant linear relationship (Table 3) between HC measures and the calibrated score of the ADOS and the age of first words. Autism symptom severity increased with HC, both in terms of residuals and genetic deviation (Table 3). Probands with larger HC had earlier onset of first words and were more likely to have a history of regression. All these patterns remained the same after excluding the 50 statistical outliers identified in the clustering analysis (Supplement: Table S5 and Figure S7)

Because HC was found to be correlated with a history of regression, we hypothesized that the pattern observed for the onset of words could be related to the mode of onset of the disorder, with children who showed a regression having a normal onset of words and an alteration of language development later. Indeed, when including regression in the model, it had a significant effect on age of onset of words ($\beta=-9.36$, $p<2.10^{-16}$) and genetic deviation was not predictive of age of first words (p=0.119, Supplement: Table S5). However we still observed a significant effect of Residual HC (Residuals, p=0.01). Of note, including regression in the models for ADOS calibrated score and IQ did not change the pattern of significant effects (Supplement: Table S5).

DISCUSSION

Converging evidence from magnetic resonance imaging and HC studies has documented that individuals with ASD have larger brains and heads than comparison subjects (3,6–16,40–43). HC has been shown to be a good measure of brain volume in children less than 6 years of age, with high correlation (0.96) between both measures at this age (44,45). Although brain volume and HC show different growth trajectories through adolescence and adulthood, HC remains an adequate predictor of brain volume after childhood (45). Therefore, abnormal HC could be an outward manifestation of underlying atypical brain development. In this study, we investigated the effects of covariates on HC, because inconsistent results from previous studies could have arisen due to the presence of confounding variables.

Heritability of body measures

The estimate of heritability for HC from the SSC data (0.63, 95% CI 0.58–0.68) is similar to that obtained from a study of parents and offspring (range of estimates 0.45 to 0.64) and is not significantly different from a study of young adult twins (0.74 95% CI 0.64–0.82) (47). Our lower estimate, compared to the twin study, could be due to the large age range in our sample, as age is controlled in the twin study. While the model used here did not accommodate the role of shared environmental factors, the similarity of its estimate to the twin study (47), which took it into account, suggests that the effects of shared environment on HC is not substantial.

Using normative data to assess macrocephaly

Applying normative data, high rates of macrocephaly were found in probands and relatives, similar to those reported in previous studies. However, genetic deviation of the “macrocephalic” probands and siblings, and the results of the mixture model analysis to define statistical outliers, revealed that only few of them were true statistical outliers. This suggests that the use of currently available normative data (e.g., (38)), as used in most HC studies in ASD, encounters several limitations.

First, correlations among height, weight, and HC were high, confirming the importance of taking height and weight into account to determine macrocephaly, as suggested by previous studies (18,22,23). Moreover, the genetic correlations and heritability of the different body measures showed that parents' body metrics have an influence on the child's HC and should be taken into account. These measures should also be considered as a simple mean to control for ancestry, which has a significant effect on HC, even for different populations from the same continent (Table 2).

One could argue that parental HC should not be taken into account because the rates of macrocephaly in unaffected relatives of individuals with autism could reflect the higher familial risk for autism (12,24). The SSC families are all simplex, which has the effect of

diminishing the genetic contribution of liability from parents to offspring, relative to a randomly chosen family (48). Thus we would expect only subtle differences in mean HC between control subjects and parents of ASD probands, similar in magnitude to the difference of means between probands and siblings.

While we cannot rule out subtle influences of this sort, our and other published data suggest the bulk of clinical outliers are a consequence of ascertainment bias and secular effect. Secular effect is well established for height (49) and Centers for Disease Control and Prevention (CDC) data (50–52) on three cohorts of infants (1929–1975, 1971–1994 and 2003–2006 periods) show a significant secular effect on HC. Indeed there is an increase of the median HC at six months of age of 7 and 11mm for boys and girls, respectively over an 80 years period covered: median of HC for boys/girls 43.7/42.4cm (1929–1975); 44/42.6cm (1971–1994); 44.4/43.5cm (2007–2010), consistent with the secular effect observed in other countries (53–56).

SSC fathers of European self-reported ancestry in this study were slightly taller (1.2cm) and heavier (2.6kg) while mothers were slightly taller (1.4cm) and lighter (−0.6kg) than the CDC comparison group (57). These differences could be related to autism risk. However, this seems unlikely because the results from the multiple trait analysis show that larger HC of probands versus their unaffected siblings is independent from an increase of height and weight. Because this study demonstrates high heritability of HC, parents with large HC will tend to have children with large HC and vice versa. The results also reveal highly significant genetic correlations between all three body metrics, demonstrating that tall parents will tend to have children who are not only tall, but with notably larger HC than average. From the genetics point of view, therefore, it makes little sense to infer outlier status for HC without taking the expected HC into account, at least when the parent and child attributes are readily available.

HC is correlated to IQ, autism symptoms and regression

ASD status is associated with larger HC, although its predictive value is limited. Individuals with increasing extreme HC showed lower IQ scores, and autism symptom severity is correlated with an increase of HC measures. The lack of adjustment for covariates and lack of power of previous studies probably explain inconsistent results regarding the correlation between HC and IQ (22,24). Because the predictive value is small, this subtle pattern can only be detected in large samples. Notably, the linear relationship between genetic deviation and clinical parameters remains significant after exclusion of statistical outliers. Taken together, these results support the hypothesis of ASD being a continuum of behaviors reflecting a continuum of changes in brain development.

These analyses also confirm the relationship between HC and a history of regression reported previously (26), which remained significant after excluding statistical outliers. The relationship with language development onset is more complex to interpret. The correlation between larger genetic deviation and earlier age of onset of words was surprising because, on average, IQ falls with increasingly positive genetic deviation and, as IQ falls, the age at first use of words increases as do ASD symptoms. Why would larger genetic deviation lead to earlier age of onset of words? A clue could reside in the positive association of genetic deviation and the probability of regression. When regression is included in the model along with genetic deviation, the relationship between genetic deviation and age of onset of words is no longer significant, showing that subjects exhibiting regression account for much of this relationship. This result is consistent with the absence of significant effect of HC on age of first phrases, which appear later in development, typically after the age for regression. Explanations for the lower age of first words for children showing regression include recall bias of age of words, regression could be more detectable when children lose use of words,

or the pattern of brain development could couple early use of words with higher probability of regression.

Implications

The results of this study show that (i) HC is a strongly heritable trait; (ii) population norms for HC would be far more accurate if covariates were taken into account; and (iii) the simplest way to obtain a predicted value for HC would be by accounting for parental HC in a model with other covariates such as age and sex. In addition these results suggest that there has been an overestimate of outliers for HC in autism. Because discovering true outliers is important, they should be identified from as much information as possible. Indeed, as currently used, normative data will classify individuals as macrocephalic far more often than is clinically appropriate, and sometimes miss others who are truly macrocephalic because their observed HC, albeit less than 2 SD based on normative data, deviates from their expected HC based on their genetic potential. Also, covariates should be taken into account when research studies evaluate genotype-phenotype relationships. These issues can be illustrated by integrating our model results and those of a recent study of *de novo* mutations identified by targeted sequencing of 44 candidate genes in the Simons collection (58), in which the authors examined HC of probands carrying *de novo* mutations. Without adjusting for covariates, they found that probands carrying protein-truncating or splice-site *de novo* *CHD8* mutations had significantly larger HC and were more frequently macrocephalic than individuals without such mutations, which they propose as a useful subphenotype. They also observed macrocephaly in probands carrying *PTEN* mutations and microcephaly in probands carrying *DYRK1A* mutations. When adjusting for covariates and parental HC, however, we find that probands carrying the *CHD8* mutations are not outliers and are not very different from their siblings (Figure 3), whereas those carrying *DYRK1A* and *PTEN* mutations are identified as statistical outliers by any measure. Our analyses show that HC is not a useful phenotype for subjects carrying *CHD8* mutations, whereas it is for subjects with severe mutations in *PTEN* and *DYRK1A* (Figure 3).

Limitations

As with any physical measures, HC is measured with error. These errors should be random, and would not be expected to bias the results, but would tend to lower correlations amongst traits. Our understanding of brain development would be strengthened by longitudinal data on HC growth. We attempted to compensate for the lack of longitudinal data by estimating the effect of age in childhood and adolescence, and the large size of the sample permitted a good approximation of this effect. There was no meaningful pattern in the residuals, by age or affection status by age (Supplement: Figure S2), suggesting that, aside from the small difference in mean HC between probands and siblings, the pattern of skull growth is similar for both, and the difference in growth likely fixed in early childhood. However, future studies would benefit from HC growth data in early childhood.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to all of the families at the participating Simons Simplex Collection (SSC) sites, as well as the principal investigators (A. Beaudet, R. Bernier, J. Constantino, E. Cook, E. Fombonne, D. Geschwind, R. Goin-Kochel, E. Hanson, D. Grice, A. Klin, D. Ledbetter, C. Lord, C. Martin, D. Martin, R. Maxim, J. Miles, O. Ousley, K. Pelphrey, B. Peterson, J. Piggot, C. Saulnier, M. State, W. Stone, J. Sutcliffe, C. Walsh, Z. Warren, E. Wijsman). We appreciate obtaining access to phenotypic data on the Simons Foundation Autism Research Initiative (SFARI) Base. Approved researchers can obtain the SSC population dataset described in this study (http://sfari.org/

resources/sfari-base), by applying at https://base.sfari.org. This work was supported by a grant from the Simons Foundation (SFARI 124827 to the investigators of the SSC Genetic Consortium); NIH K23MH082883 (SJK); NIH R01HD065272 (CL); NIH R01MH089390 (CL) and Fondamental Foundation (PC).

References

1. APA. Diagnostic and Statistical Manual of Mental Disorders, Text Revision (DSM-IVTR). 4th edition ed.. American Psychiatric Publishing; Washington, DC: 2000.
2. Courchesne E, Carper R, Akshoomoff N. Evidence of brain overgrowth in the first year of life in autism. Jama. 2003; 290:337–344. [PubMed: 12865374]
3. Aylward EH, Minshew NJ, Field K, Sparks BF, Singh N. Effects of age on brain volume and head circumference in autism. Neurology. 2002; 59:175–183. [PubMed: 12136053]
4. Hazlett HC, Poe M, Gerig G, Smith RG, Provenzale J, Ross A, et al. Magnetic resonance imaging and head circumference study of brain size in autism: birth through age 2 years. Arch Gen Psychiatry. 2005; 62:1366–1376. [PubMed: 16330725]
5. Webb SJ, Nalty T, Munson J, Brock C, Abbott R, Dawson G. Rate of head circumference growth as a function of autism diagnosis and history of autistic regression. J Child Neurol. 2007; 22:1182–1190. [PubMed: 17940244]
6. Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, et al. Autism as a strongly genetic disorder: evidence from a British twin study. Psychol Med. 1995; 25:63–77. [PubMed: 7792363]
7. Bolton P, Macdonald H, Pickles A, Rios P, Goode S, Crowson M, et al. A case-control family history study of autism. J Child Psychol Psychiatry. 1994; 35:877–900. [PubMed: 7962246]
8. Davidovitch M, Patterson B, Gartside P. Head circumference measurements in children with autism. J Child Neurol. 1996; 11:389–393. [PubMed: 8877607]
9. Lainhart JE, Piven J, Wzorek M, Landa R, Santangelo SL, Coon H, et al. Macrocephaly in children and adults with autism. J Am Acad Child Adolesc Psychiatry. 1997; 36:282–290. [PubMed: 9031582]
10. Stevenson RE, Schroer RJ, Skinner C, Fender D, Simensen RJ. Autism and macrocephaly. Lancet. 1997; 349:1744–1745. [PubMed: 9193390]
11. Fombonne E, Roge B, Claverie J, Courty S, Fremolle J. Microcephaly and macrocephaly in autism. J Autism Dev Disord. 1999; 29:113–119. [PubMed: 10382131]
12. Miles JH, Hadden LL, Takahashi TN, Hillman RE. Head circumference is an independent clinical finding associated with autism. Am J Med Genet. 2000; 95:339–350. [PubMed: 11186888]
13. Deutsch CK, Joseph RM. Brief report: cognitive correlates of enlarged head circumference in children with autism. J Autism Dev Disord. 2003; 33:209–215. [PubMed: 12757362]
14. Dementieva YA, Vance DD, Donnelly SL, Elston LA, Wolpert CM, Ravan SA, et al. Accelerated head growth in early development of individuals with autism. Pediatr Neurol. 2005; 32:102–108. [PubMed: 15664769]
15. van Daalen E, Swinkels SH, Dietz C, van Engeland H, Buitelaar JK. Body length and head growth in the first year of life in autism. Pediatr Neurol. 2007; 37:324–330. [PubMed: 17950417]
16. Ververi A, Vargiami E, Papadopoulou V, Tryfonas D, Zafeiriou DI. Clinical and Laboratory Data in a Sample of Greek Children with Autism Spectrum Disorders. J Autism Dev Disord. 2011
17. Davidovitch M, Golan D, Vardi O, Lev D, Lerman-Sagie T. Israeli children with autism spectrum disorder are not macrocephalic. J Child Neurol. 2011; 26:580–585. [PubMed: 21464237]
18. Mraz KD, Green J, Dumont-Mathieu T, Makin S, Fein D. Correlates of head circumference growth in infants later diagnosed with autism spectrum disorders. J Child Neurol. 2007; 22:700–713. [PubMed: 17641255]
19. Barnard-Brak L, Sulak T, Hatz JK. Macrocephaly in children with autism spectrum disorders. Pediatr Neurol. 2011; 44:97–100. [PubMed: 21215908]
20. Bushby KM, Cole T, Matthews JN, Goodship JA. Centiles for adult head circumference. Arch Dis Child. 1992; 67:1286–1287. [PubMed: 1444530]
21. Martins AM, Lyons Jones K. Correlation of occipitofrontal circumference and crown-rump length from birth to 15 months. Clin Dysmorphol. 1994; 3:157–159. [PubMed: 8055136]

22. Sacco R, Militerni R, Frolli A, Bravaccio C, Gritti A, Elia M, et al. Clinical, morphological, and biochemical correlates of head circumference in autism. Biol Psychiatry. 2007; 62:1038–1047. [PubMed: 17644070]
23. Chawarska K, Campbell D, Chen L, Shic F, Klin A, Chang J. Early generalized overgrowth in boys with autism. Arch Gen Psychiatry. 2011; 68:1021–1031. [PubMed: 21969460]
24. Lainhart JE, Bigler ED, Bocian M, Coon H, Dinh E, Dawson G, et al. Head circumference and height in autism: a study by the Collaborative Program of Excellence in Autism. Am J Med Genet A. 2006; 140:2257–2274. [PubMed: 17022081]
25. Rommelse NN, Peters CT, Oosterling IJ, Visser JC, Bons D, van Steijn DJ, et al. A pilot study of abnormal growth in autism spectrum disorders and other childhood psychiatric disorders. J Autism Dev Disord. 2011; 41:44–54. [PubMed: 20428954]
26. Nordahl CW, Lange N, Li DD, Barnett LA, Lee A, Buonocore MH, et al. Brain enlargement is associated with regression in preschool-age boys with autism spectrum disorders. Proc Natl Acad Sci U S A. 2011; 108:20195–20200. [PubMed: 22123952]
27. Rutter, M.; Le Couteur, A.; Lord, C. Autism Diagnostic Interview - Revised WPS Edition Manual. Western Psychological Services; Los Angeles, CA: 2008.
28. Lord, C.; Rutter, M.; DiLavore, PC.; Risi, S. Autism Diagnostic Observation Schedule WPS Edition Manual. Western Psychological Services; Los Angeles, CA: 2006.
29. Sparrow, SS.; Cicchetti, DV.; Bella, DA. Vineland Adaptive Behavior Scales—2nd edition manual. 2nd ed.. MN NCS Pearson, Inc.; Minneapolis: 2005.
30. Elliott, CD. Differential Ability Scale. Second edition.. Psychological Corporation; San Antonio, TX: 2007.
31. Wechsler, D. The Wechsler intelligence scale for children. fourth edition. Pearson Assessment; London: 2004.
32. Mullen, EM. The Mullen Scales of Early Learning. American Guidance Service Inc.; Circle Pines, MN: 1995.
33. Raven, J. Manual for Raven's Progressive Matrices and Vocabulary Scales. Research Supplement No.1: The 1979 British Standardisation of the Standard Progressive Matrices and Mill Hill Vocabulary Scales, Together With Comparative Data From Earlier Studies in the UK, US, Canada, Germany and Ireland. Harcourt Assessment; San Antonio, TX: 1981.
34. Dunn, LM.; Dunn, DM. Peabody Picture Vocabulary Test, Fourth Edition (PPVT-IV): A measure of receptive vocabulary for Standard American English. Pearson; 2007.
35. Klei L, Kent BP, Melhem N, Devlin B, Roeder K. GemTools: a fast and efficient approach to estimating genetic ancestry. 2011
36. Perez-Enciso M, Misztal I. Qxpak.5: old mixed model solutions for new genomics problems. BMC Bioinformatics. 2011; 12:202. [PubMed: 21612630]
37. Perez-Enciso M, Misztal I. Qxpak: a versatile mixed model application for genetical genomics and QTL analyses. Bioinformatics. 2004; 20:2792–2798. [PubMed: 15166025]
38. Roche AF, Mukherjee D, Guo SM, Moore WM. Head circumference reference data: birth to 18 years. Pediatrics. 1987; 79:706–712. [PubMed: 3575026]
39. Gotham K, Pickles A, Lord C. Standardizing ADOS scores for a measure of severity in autism spectrum disorders. J Autism Dev Disord. 2009; 39:693–705. [PubMed: 19082876]
40. Courchesne E, Karns CM, Davis HR, Ziccardi R, Carper RA, Tigue ZD, et al. Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. Neurology. 2001; 57:245–254. [PubMed: 11468308]
41. Piven J, Arndt S, Bailey J, Andreasen N. Regional brain enlargement in autism: a magnetic resonance imaging study. J Am Acad Child Adolesc Psychiatry. 1996; 35:530–536. [PubMed: 8919716]
42. Piven J, Nehme E, Simon J, Barta P, Pearlson G, Folstein SE. Magnetic resonance imaging in autism: measurement of the cerebellum, pons, and fourth ventricle. Biol Psychiatry. 1992; 31:491–504. [PubMed: 1581425]
43. Sparks BF, Friedman SD, Shaw DW, Aylward EH, Echelard D, Artru AA, et al. Brain structural abnormalities in young children with autism spectrum disorder. Neurology. 2002; 59:184–192. [PubMed: 12136055]

44. Lindley AA, Benson JE, Grimes C, Cole TM 3rd, Herman AA. The relationship in neonates between clinically measured head circumference and brain volume estimated from head CT-scans. Early Hum Dev. 1999; 56:17–29. [PubMed: 10530903]
45. Bartholomeusz HH, Courchesne E, Karns CM. Relationship between head circumference and brain volume in healthy normal toddlers, children, and adults. Neuropediatrics. 2002; 33:239–241. [PubMed: 12536365]
46. Weaver DD, Christian JC. Familial variation of head size and adjustment for parental head circumference. J Pediatr. 1980; 96:990–994. [PubMed: 7189556]
47. Smit DJ, Luciano M, Bartels M, van Beijsterveldt CE, Wright MJ, Hansell NK, et al. Heritability of head size in Dutch and Australian twin families at ages 0–50 years. Twin Res Hum Genet. 2010; 13:370–380. [PubMed: 20707707]
48. Klei L, Sanders SJ, Murtha MT, Hus V, Lowe JK, Willsey AJ, et al. Common genetic variants, acting additively, are a major source of risk for autism. Mol Autism. 2012; 3:9. [PubMed: 23067556]
49. Roche AF. Secular trends in human growth, maturation, and development. Monogr Soc Res Child Dev. 1979; 44:1–120. [PubMed: 503084]
50. Hamill, PVV. NCHS growth curves for children. Birth to 18 years. In: Statistics NCfH. , editor. Data from the National Health Survey. Vital and health statistics; United States: 1977.
51. Kuczmarski RJ, Ogden CL, Guo SS, et al. 2000 CDC growth charts for the United States: Methods and development National Center for Health Statistics, Vital Health Statistics. 2002; 11(246)
52. McDowell MA, Fryar CD, Ogden CL, Flegal KM. Anthropometric Reference Data for Children and Adults: United States, 2003–2006. National Health Statistics Reports. 2008; 10
53. Ounsted M, Moar VA, Scott A. Head circumference charts updated. Arch Dis Child. 1985; 60:936–939. [PubMed: 4062346]
54. Miller AK, Corsellis JA. Evidence for a secular increase in human brain weight during the past century. Ann Hum Biol. 1977; 4:253–257. [PubMed: 900889]
55. Moon JS. Secular trends of body sizes in Korean children and adolescents: from 1965 to 2010. Korean J Pediatr. 2011; 54:436–442. [PubMed: 22253640]
56. Wikland KA, Luo ZC, Niklasson A, Karlberg J. Swedish population-based longitudinal reference values from birth to 18 years of age for height, weight and head circumference. Acta Paediatr. 2002; 91:739–754. [PubMed: 12200898]
57. Fryar CD, Gu Q, Ogden CL. Anthropometric reference data for children and adults: United States, 2007–2010. National Center for Health Statistics Vital Health Statistics. 2012; 11(252)
58. Merrill RM, Richardson JS. Validity of self-reported height, weight, and body mass index: findings from the National Health and Nutrition Examination Survey, 2001–2006. Prev Chronic Dis. 2009; 6:A121. [PubMed: 19754997]
59. O'Roak BJ, Vives L, Fu W, Egertson JD, Stanaway IB, Phelps IG, et al. Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. Science. 2012; 338:1619–1622. [PubMed: 23160955]

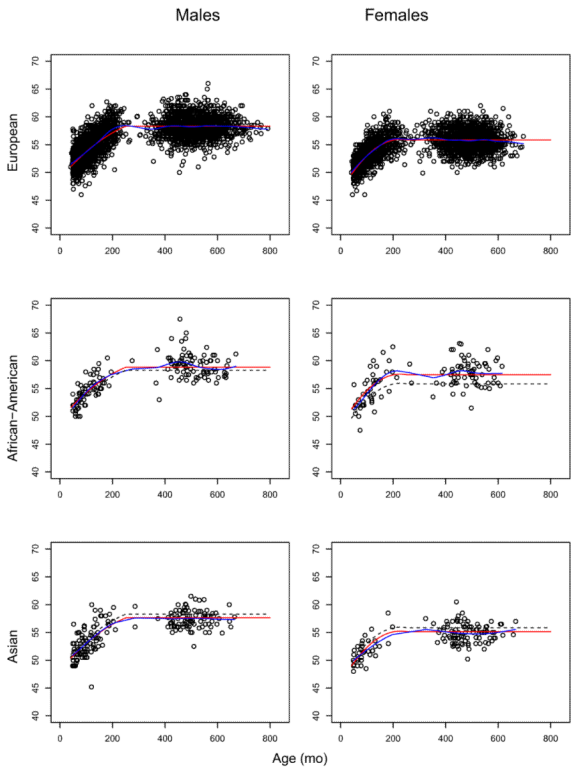

Figure 1.
Plot of head circumference versus age by sex and self-reported ancestry. Plot includes lines for a fitted smooth spline (blue) and the fitted value (red) from the model.

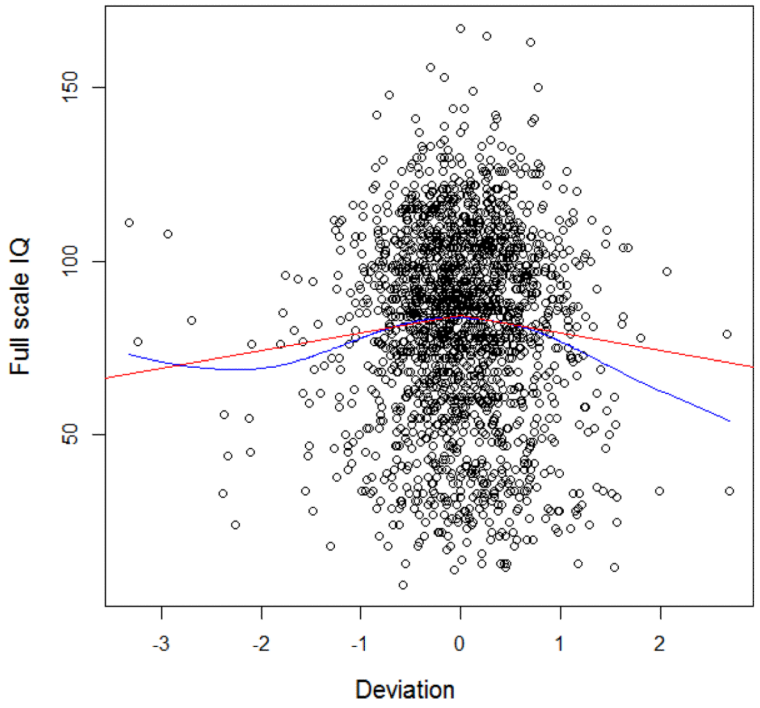

Figure 2.

Plots of full-scale IQ versus deviation of head circumference from its expected value based on a genetic model and covariates (genetic deviation). Blue line is the fitted smooth spline; red line is the fitted value from the model with the absolute value of the genetic deviation as the predictor.

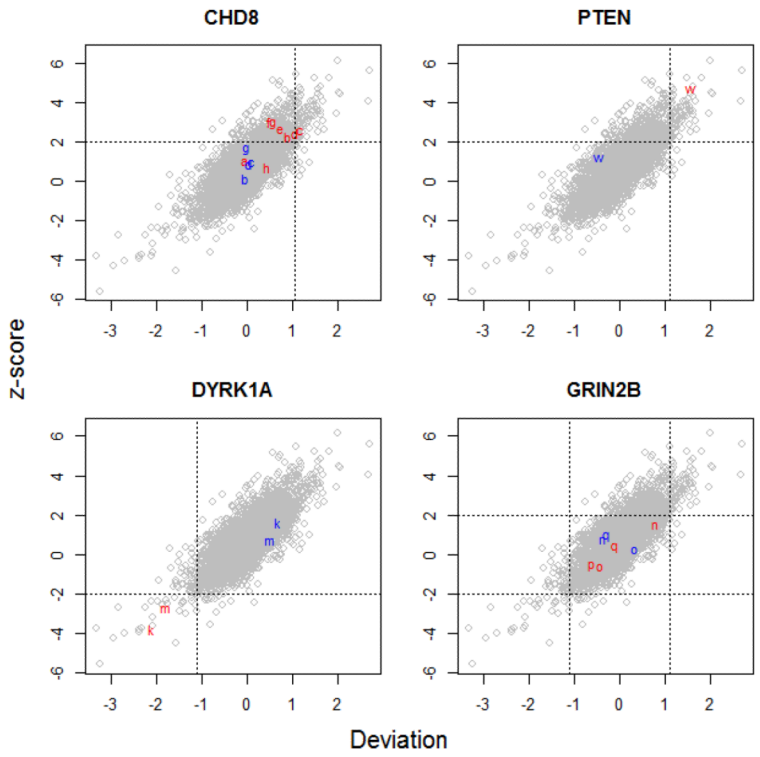

Figure 3.
Plots of z-score against deviation from expected head circumference in all individuals (grey), in individuals carrying de novo exonic mutations published in O'Roak et al (red) and their siblings (blue).

Table 1

Results from fitting head circumference as a linear function of sex, affection status, age and its squared value, height and weight.

	Regression coefficient		Standard Error		P-value	
Effect	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
mean	58.29	--				
sex	--	−1.24		0.08		3.5×10^{-56}
ASD	0.20	0.21	0.06	0.10	0.00095	0.0332
AD [a]	0.04	0.12	0.02	0.02	0.0387	5.2×10^{-10}
AD ^{2} [a]	0.00	−0.01	0.00	0.00	0.0196	2.0×10^{-9}
HT [a]	0.05	0.05	0.00	0.00	1.28×10^{-45}	4.7×10^{-30}
WT [a]	0.04	0.03	0.00	0.00	3.02×10^{-111}	1.2×10^{-62}

ASD: Autism Spectrum Disorder status; AD: Age Deviation; HT: Height; WT: Weight;

[a]All these measures were normed: age= (252 - age in month)/12 for individuals 21 and younger, 0 for all others. HT (cm) = Height observed-179.5. WT (kg) = Weight observed-95.5

Table 2

Parameter estimates for covariates from the multiple trait mixed model analysis.

		Estimate			Standard error			p-value		
Effect	**Sex**	**HT**[a]	**WT**[a]	**HC**[a]	**HT**	**WT**	**HC**	**HT**	**WT**	**HC**
Mean	179.5	95.1	**58.25**	0.16	0.36	**0.04**				
Sex		−14.4	−19.4	**−2.45**	0.23	0.52	**0.06**	0.00	0.00	**0.00**
ASD	M	−0.2	0.9	**0.23**	0.28	0.65	**0.07**	0.30	0.16	**6.5×10^{-4}**
	F	0.0	1.7	**0.27**	0.45	1.04	**0.11**	0.91	0.09	**0.01**
AD [a]	M	−0.9	−5.7	**−0.22**	0.07	0.16	**0.02**	9.1×10^{-40}	1.8×10^{-273}	**1.5×10^{-40}**
	F	1.2	−2.6	**0.11**	0.08	0.20	**0.02**	4.3×10^{-47}	5.8×10^{-39}	**1.5×10^{-7}**
AD² [a]	M	−0.2	0.1	**−0.01**	0.00	0.01	**0.00**	0.00	6.2×10^{-9}	**3.8×10^{-23}**
	F	−0.3	−0.1	**−0.03**	0.01	0.01	**0.00**	0.0000	6.7×10^{-6}	**1.4×10^{-77}**
EV1 [b]	M	50.2	39.3	**3.01**	8.25	17.46	**2.12**	1.2×10^{-9}	0.02	**0.15**
	F	76.4	68.6	**−8.67**	9.00	19.74	**2.27**	2.1×10^{-17}	5.1×10^{-4}	**1.3×10^{-4}**
EV2 [b]	M	−67.2	−76.0	**−14.57**	8.31	17.64	**2.13**	6.5×10^{-16}	1.6×10^{-5}	**8.3×10^{-12}**
	F	−80.6	−163.9	**−27.15**	9.00	19.73	**2.26**	3.3×10^{-19}	9.9×10^{-17}	**3.6×10^{-33}**
EV3 [b]	M	68.4	87.8	**9.04**	8.23	17.46	**2.10**	9.2×10^{-17}	4.9×10^{-7}	**1.7×10^{-5}**
	F	66.8	117.7	**10.70**	9.20	20.16	**2.31**	3.8×10^{-13}	5.3×10^{-9}	**3.5×10^{-6}**
EV4 [b]	M	15.7	−66.6	**−0.20**	8.19	17.25	**2.10**	0.05	1.1×10^{-4}	**0.92**
	F	8.5	−74.4	**−4.35**	8.82	19.32	**2.21**	0.34	1.2×10^{-4}	**0.048**
EV5 [b]	M	7.4	22.8	**7.80**	8.28	17.51	**2.11**	0.37	0.19	**2.1×10^{-4}**
	F	2.3	13.9	**5.17**	9.02	19.77	**2.25**	0.79	0.48	**0.02**

		Estimate			Standard error			p-value		
Effect	**Sex**	**HT**[a]	**WT**[a]	**HC**[a]	**HT**	**WT**	**HC**	**HT**	**WT**	**HC**
EV6 [b]	M	19.1	−22.1	**0.28**	8.42	18.03	**2.14**	0.02	0.22	**0.89**
	F	22.3	28.1	**1.70**	9.39	20.82	**2.34**	0.02	0.18	**0.47**
EV7 [b]	M	−17.7	0.4	**0.54**	8.85	19.29	**2.24**	0.04	0.98	**0.81**
	F	−21.8	10.4	**0.25**	9.16	20.70	**2.28**	0.01	0.62	**0.91**

HT: Height; WT: Weight; HC: Head Circumference; ASD: Autism Spectrum Disorder status; AD: Age Deviation;

[a]All these measures were normed: age= (252 - age in month)/12 for individuals 21 and younger, 0 for all others. Height (cm) = (Height observed-179.5). Weight (kg) =Weight observed-95.5;

[b]EVi - ancestry eigenvector load for EV i.

Table 3

Results from modeling clinical features as a function of head circumference (HC) traits and selected covariates.

Clinical features	HC[a] measure	b	se.b	p	R^2[b]
IQ[c]					
Verbal	Observed[d]	−0.77	0.42	0.0649	0.0018
	Residual[d]	−2.26	0.67	7×10^{-4}	0.0061
	Genetic deviation[d]	−7.07	1.84	1.2×10^{-4}	0.007
Non-Verbal	Observed[d]	−0.63	0.35	0.0695	0.002
	Residual[d]	−1.98	0.56	3.6×10^{-4}	0.0067
	Genetic deviation[d]	−6.94	1.53	5.87×10^{-6}	0.011
Full-Scale	Observed[d]	−0.79	0.37	0.035	0.002
	Residual[d]	−2.21	0.60	2.210^{-4}	0.007
	Genetic deviation[d]	−7.33	1.64	8.2×10^{-6}	0.01
ASD symptoms					
ADOS-CSS[e,f]	Residual	0.07	0.02	0.002	0.005
	Genetic deviation	0.13	0.07	0.048	0.002
Language development					
Age first words[f]	Residual	−0.56	0.20	0.005	0.004
	Genetic deviation	−1.34	0.56	0.017	0.003
Age first phrases[f]	Residual	−0.42	0.23	0.066	0.002
	Genetic deviation	−0.97	0.66	0.15	0.001
Adaptive behavior					
VABS[f,g]	Residual	0.02	0.12	0.85	0
	Genetic deviation	0.03	0.34	0.94	0
Regression[f]	Residual	0.01	0.005	0.07	-
	Genetic deviation	0.05	0.01	0.001	-

[a] Head circumference,

[b] Partial R^2

[c] Controlled for sex

[d] Head circumference was fitted as an absolute value

[e] ADOS calibrated severity score,

[f] Controlled for full scale IQ

[g] Vineland Adaptive Behavior Scales-II Composite standard score