# Adjusting Head Circumference for Covariates in Autism: Clinical Correlates of a Highly Heritable Continuous Trait

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**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 = .2 cm). 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.

**Key Words:** ASD, autism spectrum disorder, body metrics, genetic ancestry, head circumference, IQ

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 SD) in ASD compared with the general population (6–16). However, some studies suggested that the use of national normative samples as control data could

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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 yet clear. Some authors suggest that macrocephaly is an endophenotype pointing toward 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 multistep analysis of 2644 Simons Simplex Collection (SSC) families, using a mixed model that provided 1) estimates of covariates' effects and 2) 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 test accurately whether 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 and Materials

### Sample

The 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 Supplement 1 for inclusion and exclusion criteria.

Each SSC center designated one person to measure all participants HC (for protocol, see Supplement 1), 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—Western Psychological Services (WPS) edition (27), Autism Diagnostic Observation Schedule—WPS edition (ADOS-WPS) (28), Vineland Adaptive Behavior Scale—2nd edition (VABS-II) (29). IQ was evaluated either with the Differential Ability Scales—2nd edition (30), Wechsler Intelligence Scale for Children—4th edition (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 (34). The SSC families were genotyped on the Illumina Infinium 1Mv3 (duo) or the Illumina Infinium 1Mv1 microarrays (both Illumina, Inc, San Diego, California), providing genome wide genotype data to estimate genetic ancestry. After editing the data for missing information, 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 1).

### 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, and 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 single nucleotide polymorphism genotypes (5156 with minor allele frequency $>.01$, noncompletion rate $<.002$), 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) male subjects equaled to 0. Self-reported ancestry showed reasonable agreement with genetically inferred ancestry (Table S4 and Figure S1 in Supplement 1). 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 Asian groups, likely Chinese and Indian ancestry; and 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,