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Head Circumferences in Twins With and Without Autism Spectrum Disorders

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Abstract

To determine the genetic relationship between head circumference (HC) and Autism Spectrum Disorders (ASDs). Twin pairs with at least one twin with an ASD were assessed. HCs in affected and unaffected individuals were compared, as were HC correlations in monozygotic and dizygotic pairs. 404 subjects, ages 4–18, were included. 20 % of males and 27 % of females with an ASD had macrocephaly. Unaffected co-twins showed similar rates (15 % of males and 22 % of females). Statistical analysis revealed no significant difference in HCs between affected and unaffected twins. Twins with ASDs and unaffected co-twins have similar HCs and increased rates of macrocephaly. Correlations demonstrated partial inheritance of HCs. Thus, macrocephaly may represent an endophenotype in ASDs.

Keywords

Macrocephaly; Head circumference; Twins; Endophenotype; Genetics

Introduction

Twin studies suggest that a combination of genetic and environmental factors lead to the development of the Autism Spectrum Disorders (ASDs) (Bailey et al. 1995; Folstein and Rutter 1977; Steffenburg et al. 1989; Hallmayer et al. 2011). Recent genetic studies have identified rare genetic mutations in about 10–20 % of cases. However, genome wide association studies (GWAS) and linkage analysis studies have not uncovered any specific common genetic variant associated with ASDs (Abrahams and Geschwind 2008; Anney et al. 2010). The lack of success in identifying common causal genetic variants is likely due to ASDs developing from several different pathways determined by combinations of multiple genes and environmental factors.

In his first description of autism in 1943, Leo Kanner noted that 5 of the 11 children had relatively large heads (Kanner 1943). Since that time, multiple studies have established macrocephaly as the most common physical finding amongst children with ASDs, with

prevalence estimates ranging from 11 % (van Daalen et al. 2007) to 29.7 % (Woodhouse et al. 1996). Changes in head circumference associated with autism reflect abnormal brain growth (Courchesne et al. 2003). Macrocephaly is not present at birth in children later diagnosed with autism, but results from an increased growth rate in early childhood. This is subsequently followed by decreased growth velocity in later childhood and adolescence (Courchesne 2004; Herbert 2005). Macrocephaly is present at a higher rate not only in individuals with ASDs, but also in their first degree relatives (Fidler et al. 2000; Miles et al. 2000; Lainhart et al. 2006). Constantino et al. (2010) found higher correlations for HC growth rate between sibs in which at least one of the siblings had been diagnosed with an ASD (ICC=0.63) compared to control siblings without ASDs (ICC = 0.26), indicating that HC growth velocity may be familial. These studies suggest that macrocephaly and/or increased head growth velocity may represent an endophenotype within ASDs. An endophenotype refers to a heritable biomarker that co-segregates with illness in families, but is present in family members both with and without the illness. Gottesman and Gould have described the following criteria for defining endophenotypes in psychiatric genetics: “(1) The endophenotype is associated with illness in the population. (2) The endophenotype is heritable. (3) The endophenotype is primarily state-independent (manifests in an individual whether or not illness is active). (4) Within families, endophenotype and illness co-segregate.” and for diseases with complex inheritance, “(5) The endophenotype found in affected family members is found in nonaffected family members at a higher rate than in the general population” (Gottesman and Gould 2003).

A related question is whether macrocephaly denotes a different subtype of autism. Investigators have questioned whether macrocephaly is associated with certain characteristics in individuals with ASDs. Sacco et al. (2007) found that increased HC in children with autism was associated with lower levels of adaptive functioning and more social difficulties, but higher intelligence quotient (IQ) and better language abilities. Similarly, Davidovitch et al. (1996) found ASD individuals with macrocephaly to have lower adaptive functioning. Lainhart et al. (2006) also found increased HC to be associated with more social difficulties. However, they did not find any correlation between HC and IQ in autistic children, whereas HC did correlate with IQ in their controls. Deutsch and Joseph (2003) also found no association between HC and IQ, language abilities, or executive functioning. They did, however, find that increased HC correlated with a greater nonverbal IQ-verbal IQ difference in individuals with autism.

To better clarify the genetic relationship between HC and ASDs, we employed a twin study design. Monozygotic (MZ) twin pairs have identical genomes, and dizygotic (DZ) pairs share on average half of the genome. Differences between MZ and DZ pairs in the co-twin correlation of specific phenotypic features, such as HC, allow estimation of the contribution of genetic factors to the phenotype. Using a large sample of twin pairs where at least one twin carried an ASD diagnosis, we sought to determine the rates of macrocephaly in both affected and unaffected co-twins. We also investigated whether HC correlates with certain measures of cognitive and behavioral functioning, as well as the degree to which HC is heritable in the setting of ASDs. These investigations were undertaken in order to help ascertain whether macrocephaly represents an endophenotype or subtype of ASD. This study was approved by the Institutional Review Board at Stanford University and the California Health and Human Services Agency, Committee for the Protection of Human Subjects.

Methods

Participants

Subjects were identified from the California Department of Developmental Services (DDS) and Regional Centers and were included in the California Autism Twin Study (CATS), as described in detail in Hallmayer et al. 2011. In brief, charts from twin births occurring in California between 1987 and 2004 were reviewed. Twin pairs were selected for inclusion in the study if the following criteria were met: (1) at least one child in the pair had a documented ASD diagnosis (2) maternal residence was in California at the time of delivery and both co-twins resided in California at the time of study enrollment, and (3) at least one parent had sufficient verbal and reading ability in English or Spanish to complete interviews and checklists. Exclusion criteria included documented neurogenetic conditions associated with autism (e.g., fragile X syndrome, Down syndrome, tuberous sclerosis and neurofibromatosis) and mental age less than 18 months (Hallmayer et al. 2011).

Initially, 1,157 twin pairs with at least one child with documented clinical evidence of an ASD were identified. Of these, 533 families agreed to be contacted, and assessments were completed on 212 twin pairs from families who gave their informed consent prior to participation. Following assessments, 10 pairs were found to have neither twin meet criteria for an ASD based on the ADI-R and ADOS and were removed. The analytic sample included 404 subjects (202 twin pairs) ranging from ages 4–18 at time of assessment.

Diagnostic Assessments

Cognitive and behavioral assessments of twin pairs were carried out by the Autism Genetic Resource Exchange (AGRE) in Southern California and Stanford University in Northern California. To verify clinical diagnoses and establish research diagnoses, parents were administered the Autism Diagnostic Interview—Revised (ADI-R) (Lord et al. 1994; Rutter et al. 2003) and the Autism Diagnostic Observation Schedule (ADOS) (Lord et al. 1999; Lord et al. 2000). With parental consent, both the ADI-R and ADOS assessments were videotaped for reliability purposes. All assessors attended official research training and achieved reliability with the site psychologists. Consensus reviews were performed on difficult cases and randomly selected cases for ongoing reliability.

In addition to the ADI-R and ADOS, verbal and nonverbal cognitive abilities were assessed using either the Stanford-Binet, 5th Edition (SB5) (Roid 2003) or the Mullen Scales of Early Learning (MSEL) (Mullen 1995). Assessors attempted to administer the SB5 to all twins. For children unable to achieve a basal score on the SB5 due to either behavioral or cognitive difficulty, the MSEL was administered. Using the MSEL, Nonverbal IQ (NVIQ) was calculated by averaging the age-equivalent scores from the Visual Reception and Fine Motor domains, and dividing the resulting age equivalent by the child's chronological age to yield a percentage score comparable to a standardized IQ score. Similarly, Verbal IQ (VIQ) was calculated using the Receptive Language and Expressive Language domains. Full Scale IQ (FSIQ) for subjects unable to fully complete the SB5 were calculated as averages of the estimated VIQ and NVIQ. Of the 404 individuals, 366 fully completed the SB5, 7 completed the nonverbal section of the SB5 (3 of whom also completed the verbal section of the MSEL), and 1 completed only the verbal section of the SB5. An additional 10 individuals completed both the nonverbal and verbal sections of the MSEL. The remaining 20 individuals were not able to complete any sections of either the MSEL or the SB5. Level of functioning was assessed using the Vineland Adaptive Behavior Scales (VABS) (Sparrow et al. 2005), a standardized, semi-structured parent interview frequently used to measure adaptive abilities in children with autism (Freeman et al. 1999; Kraijer 2000). The Social Responsiveness Scale (SRS), a 65-item parent report measure was administered to assess

social traits (Constantino 2002; Constantino et al. 2003). Finally, verbal ability was further quantified using the Peabody Picture Vocabulary Test (PPVT), an individually administered test of receptive language and vocabulary (Dunn et al. 2006).

Physical Measurements

HCs were measured at the time of assessment using a detailed protocol designed for the study. With the child observing, the examiner first modeled measuring their own head and then the parent's head in order to familiarize the child with the procedure. With the child facing the examiner, the examiner then wrapped the tape measure around the largest part of the head starting above the left ear, around and over the protuberance of the back of the head, around to the right side of the head (above the right ear) and then across the forehead just above the eye brows and back to the left side of the head. The measurement was repeated three times, and the maximum head circumference was recorded (Gundy 2001; Menounou 2011). For height and weight, parents completed a medical history questionnaire including current height and weight for each twin.

Diagnostic Classes

Diagnostic classification was determined based on ADI-R and ADOS results. Overall, individuals were considered "affected" if they met criteria for a broad phenotype ASD as described in Hallmayer et al. 2011 using published criteria for combined ADI-R and ADOS subscores (Risi et al. 2006). In brief, individuals were considered broad phenotype if they failed to meet strict autism criteria for both the ADOS and ADI-R, but met blended criteria according to the following combinations:

1. Met criteria for autism spectrum on the ADOS and met full criteria on the ADI-R for autism on all of the domains
2. Met criteria for either autism or autism spectrum on the ADOS and met the ADI-R autism cutoffs on the domains of social reciprocity and either communication or restricted, repetitive behaviors
3. Met criteria for either autism or autism spectrum on the ADOS and scored within one point of meeting the ADI-R autism cutoffs on the social and communication domains
4. Met criteria for either autism or autism spectrum on the ADOS and met the autism cutoff on either the social or communication domains of the ADI-R and scored within two points on the other.

"Proband" status was determined independent of study assessments and based on the presence of an ASD documented in the DDS electronic file or Regional Center client's chart. In cases where both twins were documented to have an ASD in the clinical files, both twins were designated probands. Twin pairs were considered "ASD concordant" if both individuals met ASD research criteria. In contrast, "ASD discordant" pairs consisted of one affected and one unaffected twin. Zygosity was determined by DNA analysis (Hallmayer et al. 2011).

Data Analysis

Rates of Macrocephaly—Rates of macrocephaly (defined as HC > 97 % according to United States Head Circumference Growth Reference Charts Appendix values (Rollins et al. 2010)) were determined. Rates of macrocephaly were compared between children younger than 12 years and children 12 years and older using Chi square analysis. The cut-off age of 12 years was chosen due to a previous study showing abnormally slow growth in brains of children with autism between the ages of 4 and 11.5 years (Carper et al. 2002). In twin pairs

with at least one twin with macrocephaly, Fisher's exact test was employed to compare rates of macrocephaly in ASD concordant and ASD discordant groups.

HC Comparisons Between Affected and Unaffected—Paired samples *t* tests were performed comparing HCs in sex concordant, but ASD discordant twin pairs. Using linear regression with age as the independent variable, HC residuals were defined as the difference of the actual HC from the HC that was predicted by the regression function including age. We then compared HC residuals between affected and unaffected groups in sex concordant twin pairs.

Within pair differences in both HC and HC residuals were calculated. For ASD discordant pairs, the HC or HC residual of the unaffected twin was subtracted from that of the affected twin. In sex concordant twin pairs, we compared the absolute values of these differences in ASD concordant groups to those in ASD discordant groups using Chi square analysis. Additionally, mean differences in HC for sex concordant, ASD discordant groups were correlated with age.

HC Correlation with Height/Weight, and Cognition, Adaptive Behavior, and Social Traits—Height and weight percentiles were based on the Center for Disease Control 2000 Growth Charts (Kuczmarski et al. 2000). Spearman's correlations between height, weight, and HC percentiles were calculated. The relationship between HC and quantitative variables including FSIQ, NVIQ, VIQ, PPVT total score, VABS composite score, and SRS total was investigated with partial correlations controlling for age and gender.

Genetic Factors—*t* Tests were used for comparison of between-twin differences in HC and HC residuals in MZ compared to DZ twins. Additionally, correlations were used to describe between-twin similarities in HCs. Because ASD discordant twins could be stratified with the affected twin as Twin 1 and the unaffected twin as Twin 2, Pearson's interclass correlations were used for ASD discordant twins, while intraclass correlations were used for ASD concordant twins. Correlation coefficients for sex concordant MZ twins were contrasted with correlation coefficients for sex concordant DZ twins in both affected and unaffected groups using Fisher's *r*-to-*z* transformation. The contribution of genetic factors to head circumference in children with autism (heritability on the background of autism, or "overlying heritability") was then estimated using Falconer's formula for estimating heritability (broad sense) in twin studies:

$H = 2(r(MZ) - r(DZ))$ where *H* represents heritability, *r*(MZ) is the intraclass correlation coefficient for MZ twins and *r*(DZ) is the interclass correlation coefficient for DZ twins (Falconer and MacKay 1996).

Results

Rates of Macrocephaly

Twin pairs consisted of 48 monozygotic male (MZM) pairs, 8 monozygotic female (MZF) pairs, 50 dizygotic male (DZM) pairs, 14 dizygotic female (DZF) pairs, and 82 sex discordant (SD) pairs. Based on diagnostic assessments, 53 pairs were ASD concordant (35 MZ and 18 DZ), and 149 pairs were ASD discordant (21 MZ and 128 DZ). Mean age was 9.7 years, and the distribution is shown in Fig. 1.

Overall, 83/404 twins (21 %) met criteria for macrocephaly (see Table 1). For individuals with ASD, 20 % of males and 27 % of females were macrocephalic. Additionally, 75 % of males and 84 % of females had HCs greater than the 50th percentile. Unaffected co-twins

also had greater than expected percentages of macrocephaly compared to the general population (3 % by definition). In the unaffected co-twins, 15 % of males and 22 % of females had macrocephaly, while 83 % of males and 71 % of females had HCs in the top half of norms. In contrast to macrocephaly, rates of microcephaly in this sample were much closer to the expected 3 % in the general population. Only 6 individuals (1.5 %) were microcephalic, and of these 6, only 1 was unaffected.

Chi square analysis demonstrated no significant differences in rates of macrocephaly in affected versus unaffected individuals for the entire sample. Nor were there any significant differences in rates of macrocephaly ($p > 0.05$) when the sample was subgrouped according to gender alone (males vs. females) or zygosity alone (MZ vs. DZ). Because of the relatively small number of MZFs, Ns were too small to analyze differences based on gender and zygosity together. Among twin pairs with at least one macrocephalic twin, the number of twin pairs concordant for macrocephaly was similar for ASD concordant and ASD discordant pairs in both MZ and DZ subgroups ($p > 0.05$, Fisher's exact test). In affected and unaffected subgroups, rates of macrocephaly were similar for subjects <12 years and subjects ≥ 12 years (Chi square = 0.011, $p > 0.05$). Because macrocephaly rates were similar, further analyses did not divide groups according to age.

Comparing HC in Affected and Unaffected Individuals

Comparing HC among sex concordant, but ASD discordant twin pairs, showed no significant differences in HC between affected and unaffected twins (Table 2). In 36 of the 72 pairs, the affected twin had a larger HC, in seven pairs HCs were equal, and in 29 pairs the unaffected twin had the larger head. For sex concordant pairs, the mean of the absolute values of differences in HC between ASD discordant twins (1.32 cm) was not significantly larger than the mean in ASD concordant pairs (1.22 cm, $p = 0.68$) (Table 3).

Age correlated significantly with HC in both affected and unaffected twins ($r = 0.59$ affected, $p < 0.001$ and $r = 0.67$ unaffected, $p < 0.001$). These correlations between the affected and unaffected groups were not significantly different ($z = -1.35$, $p > 0.05$). Therefore, linear regression using age as an independent variable was performed for the entire sample as a whole, as opposed to independently for affected and unaffected groups. We then examined whether residual differences in HC between ASD concordant and ASD discordant twin pairs differed significantly among sex concordant pairs. If affected individuals were to have a growth trajectory consisting of rapid acceleration followed by deceleration, one would expect the differences in HC residuals in ASD discordant pairs to be greater than differences in ASD concordant pairs. However, univariate analysis comparing the absolute values of HC residual differences in these groups demonstrated no significant difference ($F = 0.03$, $p > 0.05$). Furthermore, the differences in HC between sex concordant, ASD discordant twin pairs did not correlate significantly with age ($r = 0.04$, $p > 0.05$).

HC Correlation with Height and Weight—In general, HC correlated significantly with height and weight for the sample as a whole, as well as for all males, all females, all affected twins, and all unaffected twins. Correlation coefficients ranged from 0.58 to 0.72 for HC with height and from 0.69 to 0.73 for HC with weight. Overall, 22 (9 %) of the 254 children for whom heights were available had heights greater than the 97th percentile, and 85 (34 %) had heights greater than the 75th percentile. Forty-three (14 %) of the 306 twins with weights available had weights greater than the 97th percentile, and 124 (41 %) had weights greater than the 75th percentile. Of the 50 macrocephalic children with available height, 11 (22 %) were above the 97th percentile, and 27 (54 %) were above the 75th percentile for height. Twenty-one (35 %) of the 60 macrocephalic children with weights available were greater than the 97th percentile, and 47 (78 %) were over the 75th percentile for weight.

HC Correlation with Cognition, Adaptive Behavior, and Social Traits—For affected individuals, HC showed small negative correlations (r 's ranging from -0.184 to -0.126 , $p < 0.05$) for FSIQ, NVIQ, VIQ, PPVT, and VABS, but no significant correlation with the SRS. For unaffected individuals, HC did not correlate significantly with any of the measures (Table 4). In macrocephalic children, HC correlated negatively with FSIQ, VIQ and PPVT and positively with SRS ($p < 0.05$) (note that higher scores on the SRS indicate more impairment.)

Genetic Factors—Among sex concordant pairs, there was no significant difference between MZ and DZ twin pairs in the rates of macrocephaly concordance (Table 5). The mean between-twin difference in head size in MZ ASD discordant twins [mean HC difference = 0.0024 cm (less than 1 mm), subtracting the HC of the unaffected twin from the HC of the affected twin] was somewhat smaller than the mean difference in DZ ASD discordant twins (mean HC difference = 0.23 cm) (Table 3). However, this comparison did not reach significance ($p > 0.05$). Similarly, there were also no significant differences between MZ and DZ groups in either absolute values of HC differences, HC residuals, or the absolute values of HC residuals. However, the MZ differences were consistently, though non-significantly, smaller than the DZ differences.

Similarly, between-twin correlations in HCs were somewhat larger (though non-significantly larger) in MZ twins as compared to DZ twins. Regardless of affection status, HCs of co-twins correlated significantly for all sex concordant twin pairs (Table 6). Correlations for HCs were approximately 20–35 % greater in MZ as compared to DZ twins indicating that HC is partially heritable, but not entirely dependent on genetics. Calculating heritability for HC in all sex concordant twin pairs demonstrated “overlying heritabilities” ranging from 0.32 to 0.38 for ASD discordant and ASD concordant pairs.

Discussion

The rates of macrocephaly in this twin study ranged from 20 to 27 %, consistent with reports of macrocephaly among singletons with ASD (Woodhouse et al. 1996). Interestingly, co-twins who did not meet criteria for ASD, had similar rates of macrocephaly. Approximately 50 % of affected twins had measurably larger heads than their unaffected sex concordant co-twins, but nearly as many sex-concordant, ASD discordant pairs had unaffected twins with larger heads. In MZ and DZ subgroups, concordance rates of macrocephaly in twin pairs concordant for ASD were not different from those in pairs discordant for ASD. Paired samples t tests produced no significant differences in HC between affected and unaffected twins.

Although the results presented focus on affected and unaffected as defined by broad ASD criteria, it should be noted that rates of macrocephaly were similar when calculated using narrowly defined or “strict” autism criteria with full autism criteria being met on both the ADOS and ADI-R. For this more narrowly defined phenotype, rates of macrocephaly remained statistically similar with 37/132 (22 %) of unaffected twins and 46/189 (24 %) having macrocephaly. For males, 14/73 (19 %) unaffected males and 39/166 (23 %) affected males were macrocephalic, while 23/96 (24 %) unaffected females and 7/30 (23 %) affected females had macrocephaly. Similarly, paired samples t tests continued to show no significant difference in HCs for narrowly defined affected twins (mean = 54.29 cm) as compared to unaffected twins (mean = 54.23 cm) in sex-concordant, ASD discordant pairs.

As shown in Table 3, mean differences in HC and HC residuals for sex concordant twin pairs were not significantly different for ASD concordant twin pairs as compared to ASD discordant pairs. Furthermore, all groups demonstrated increased rates of macrocephaly

regardless of age or affected status. HC and age were significantly correlated in both affected and unaffected co-twins, and the difference in HC between ASD discordant twins did not correlate with age. Thus, the above findings demonstrate that affected and unaffected twins in this sample appear to have both similar HCs, as well as similar head growth trajectories. Taken together, these results suggest that macrocephaly may be a marker for a specific endophenotype within ASDs: Macrocephaly is associated with autism; HC is heritable; and macrocephaly is found in unaffected co-twins at a higher rate than in the general population. Results also demonstrate that whatever the process leading to macrocephaly may be, this process alone is not sufficient for the development of autism.

In affected individuals, HC correlated negatively with IQ, as well as the PPVT and VABS. HC did not correlate with IQ or other quantitative measures in unaffected individuals. Other studies have demonstrated a positive correlation between HC and/or brain volume and IQ in typical individuals (Reiss et al. 1996; Wickett et al. 2000; Ivanovic et al. 2004; Lainhart et al. 2006). Thus, it is possible that the unaffected co-twins represent an intermediate phenotype between affected individuals whose HCs correlate negatively, and unrelated, unaffected individuals whose HCs correlate positively with IQ.

Correlations within sex-concordant pairs were approximately 20–35 % greater in MZ compared to DZ pairs indicating partial inheritance for HC in twins where at least one twin has an ASD. These “overlaying heritabilities” for HC, or heritability in twin pairs with at least one twin with an ASD, ranged from 0.32 to 0.38, which is similar to a previously reported heritability of 0.47 for individuals with autism and other developmental disabilities (Fidler et al. 2000). However, these rates are much smaller than heritabilities reported for HC in typically developing children and adults, which range from 0.75 to 0.9 (Smit et al. 2010). Environmental factors that affect HC may be influencing those with a genetic predisposition to ASDs more so than those without genetic susceptibility.

Bolton et al. (2001) showed that macrocephaly is associated with an increased risk of ASD (OR = 5.44, 95 % CI 1.11–52.15) in a cohort study of boys born with idiopathic macrocephaly. However, macrocephaly itself is neither sufficient, nor necessary for the development of an ASD. Genetic factors have been identified in some, but not all, children with macrocephaly and autism; yet none are specific to the development of ASDs. For example, variations in the tumor suppressor gene *PTEN* have been associated with both autism and macrocephaly. However, only a subset of individuals with *PTEN* variations have ASDs, and only about 8 % of individuals with ASDs and macrocephaly have variations in *PTEN* (Varga et al. 2009). Deletions in chromosome 17q12 (Moreno-De-Luca et al. 2010), mutations in the small GTPase *RAB39B* gene (Giannandrea et al. 2010), and mutations in the *NSD1* gene (Buxbaum et al. 2007) are other genetic variations that have all been associated with ASDs and macrocephaly.

Because autism is a complex and heterogeneous disorder, defining endophenotypes may help clarify underlying biological processes that lead to behavioral symptoms. Identifying endophenotypes such as macrocephaly may significantly advance studies seeking genetic risk loci by narrowing the variability in subject samples. Furthermore, endophenotypes provide opportunities for investigating gene-environment interactions, and ultimately they improve the overall understanding, diagnosis, treatment, and prevention of ASDs.

There are several limitations to this study. First, heights and weights were not collected at the time of assessment, and missing data from questionnaires for these values limited statistical power. Thus, this sample is not ideal for determining whether the macrocephaly seen in this study was isolated macrocephaly or part of a broader generalized macrosomy as has been suggested by some studies (van Daalen et al. 2007; Sacco et al. 2007; Mraz et al.

2007). Based on parent report heights and weights, correlations between HC and heights and weights were somewhat greater in this sample as compared with the general population as reported in Geraedts et al. (2011) which showed r 's ranging from 0.37 to 0.59 for height and 0.44–0.63 for weight in a reference group of Dutch children. Most of the children with macrocephaly in our sample were also relatively large based on height and weight. Furthermore, this sample overall has a somewhat overrepresentation of children with heights greater than both the 97th and 75th percentiles. Taken together, these data may suggest that an endophenotype may be more consistent with a generalized macrosomy as opposed to a specific macrocephaly.

A second limitation of this study is that only twins who had been referred to their Regional Centers were identifiable during recruitment. Thus, a selection bias favoring lower functioning probands may exist, as higher functioning individuals may not have been referred to their Regional Centers. However, it should be noted that the sample studied did include 20 ASD discordant pairs in which the affected twin met autism spectrum, but not strict autism criteria. Furthermore, this study found similar rates of macrocephaly in affected and unaffected co-twins and limited correlation between HC and level of functioning, suggesting that inclusion of a greater number of high functioning individuals should not significantly affect results.

A third limitation is that the sample did not include unrelated typically developing controls for comparison. A recently published epidemiologic sample failed to find significant differences in the rates of macrocephaly between children and controls (Barnard-Brak et al. 2011). The children in this study were considerably younger than the children in our sample as time points were measured at 9, 24, and 36 months. Selection bias must also be considered with regards to the families who chose to participate in the study and those who did not. As reported in Hallmayer et al. (2011), the two groups were comparable for most variables, except that the age of the twins included in the genetic analyses was on average slightly older, birth weight of males was somewhat higher, and their mothers and fathers were, on average, slightly more educated, and more likely to be white and less likely to be African American. There was no significant difference in parental age, gestational age, birth weight in females, or gender. There was also no significant difference in the proportion of concordance for documented ASD diagnoses (Hallmayer et al. 2011). Although the average birth weight for males in this sample (2,532 g) was slightly higher than that of the male twins whose families chose not to participate in the study (2,442 g, $p < 0.05$), the average birth weight for males in the study was still well within the range of normal for twin births. (For females in the study, average birth weight was 2,436 and 2,381 g respectively.) The average gestational age for both the twins who participated in the study, and those who did not was 36 weeks (Hallmayer et al. 2011). The average birth weights for twins in this sample are all near the reported average birth weights for 36 week twins (2,491 g for males and 2,411 g for females) and fall close to the 50th percentile on growth charts for male and female twin births (Arbuckle et al. 1993). Also regarding size, it should be noted that while recent US norms were used to define macrocephaly, the sample was derived from twins in California, a state with significantly higher numbers of minorities. Finally, although the sample size is relatively large, subject numbers are still too small to allow for partial correlations that account for both age and gender, and numbers for female-only twin pairs remain limited.

Conclusions

Findings from this study support a prevalence of macrocephaly ranging from 20 to 27 % in children with ASDs, similar to previous reports. However, these rates are similar and not significantly higher than the prevalence of macrocephaly in their unaffected co-twins. Thus,

the developmental change responsible for macrocephaly is itself not sufficient for causing ASDs. HC correlated negatively with IQ in twins affected with ASDs, but there was no correlation between IQ and HC in unaffected co-twins. Therefore, unaffected co-twins may represent an intermediate phenotype between affected individuals whose HCs correlate negatively, and unrelated, unaffected individuals whose HCs correlate positively with IQ. Finally, between twin correlations for HC comparing MZ and DZ sex concordant twin pairs suggest that HC is partially inherited. Given that HC is heritable, increased head size is associated with ASDs, and macrocephaly is found in unaffected co-twins at a higher rate than in the general population, macrocephaly may represent an endophenotype in ASDs.

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Fig. 1.
Age distribution

Table 1

Rates of macrocephaly

	All twins			# Microcephalic			Affected twins			Unaffected co-twins		
	N	# Macrocephalic	# >50 %	N	# Macrocephalic	# >50 %	N	# Macrocephalic	# >50 %	N	# Macrocephalic	# >50 %
All	404	83	(21 %)	307	(76 %)	6 (1 %)	255	54	(21 %)	194	(76 %)	113 (76 %)
Males	278	53	(19 %)	213	(77 %)	3 (1 %)	218	44	(20 %)	163	(75 %)	50 (83 %)
Females	126	30	(24 %)	74	(59 %)	3 (2 %)	37	10	(27 %)	31	(84 %)	63 (71 %)
MZ	112	20	(18 %)	83	(74 %)	1 (1 %)	91	17	(19 %)	67	(74 %)	16 (76 %)
DZ	292	63	(22 %)	224	(77 %)	5 (2 %)	164	37	(23 %)	127	(77 %)	97 (76 %)
MZM	96	19	(20 %)	71	(74 %)	1 (1 %)	80	16	(20 %)	58	(73 %)	13 (81 %)
MZF	16	1	(6 %)	12	(75 %)	0 (0 %)	11	1	(9 %)	9	(82 %)	3 (60 %)
DZM	100	12	(12 %)	79	(79 %)	2 (2 %)	59	7	(12 %)	44	(75 %)	35 (85 %)
DZF	28	9	(32 %)	26	(93 %)	1 (4 %)	18	5	(28 %)	17	(94 %)	9 (90 %)

MZ monozygotic, *DZ* dizygotic, *MZM* monozygotic males, *DZM* dizygotic males, *MZF* monozygotic females, *DZF* dizygotic females

Table 2

Paired *t* tests: sex-concordant, ASD discordant twins

	<i>N</i>	Affected twins Mean HC (cm)	Unaffected twins Mean HC (cm)	<i>t</i>	<i>p</i>
All	72	54.35	54.19	0.775	(0.44)
All Males	57	54.39	54.39	-0.032	(0.97)
All Females	15	54.23	53.42	1.43	(0.17)
MZM	16	55.01	55.18	-0.212	(0.84)
DZM	41	54.11	54.09	0.09	(0.93)
MZF	5	53.41	53.12	0.58	(0.59)
DZF	10	54.64	53.57	1.31	(0.22)

MZM monozygotic males, *DZM* dizygotic males, *MZF* monozygotic females, *DZF* dizygotic females

Table 3

Between-twin head circumference differences

	N	Mean (cm)	SD	SE Mean	t	p
Comparing ASD discordant to ASD concordant						
HC difference (affected HC—unaffected HC for ASD discordant pairs)						
ASD discordant	72	0.16	1.78	0.21	−0.94	(0.35)
ASD concordant	48	0.47	1.77	0.25		
Absolute value of HC difference						
ASD discordant	72	1.32	1.20	0.14	0.42	(0.68)
ASD concordant	48	1.22	1.35	0.20		
HC residual difference (affected HC residual—unaffected HC residual for ASD discordant pairs)						
ASD discordant	72	0.06	0.79	0.09	−1.279	(0.20)
ASD concordant	48	0.25	0.79	0.11		
Absolute value of HC residual difference						
ASD discordant	72	0.58	0.54	0.06	0.16	(0.87)
ASD concordant	48	0.56	0.60	0.09		
Comparing MZ to DZ						
Sex concordant, ASD discordant twins						
HC difference						
Monozygotic	21	0.0024	1.52	0.33	−0.487	(0.63)
Dizygotic	51	0.23	1.89	0.26		
Absolute value of HC difference						
Monozygotic	21	1.09	1.03	0.23	−1.02	(0.31)
Dizygotic	51	1.41	1.26	0.18		
HC residual difference						
Monozygotic	21	−0.04	0.69	0.15	−0.67	(0.51)
Dizygotic	51	0.10	0.83	0.12		
Absolute value of HC residual difference						
Monozygotic	21	0.49	0.47	0.10	−0.91	(0.37)
Dizygotic	51	0.62	0.57	0.08		
Sex concordant, ASD concordant twins						
HC difference						

	N	Mean (cm)	SD	SE Mean	t	p
Monozygotic	35	0.16	1.50	0.25	-2.054	(0.05)
Dizygotic	13	1.30	2.19	0.61		
Absolute value of HC difference						
Monozygotic	35	1.00	1.12	0.19	-1.875	(0.07)
Dizygotic	13	1.80	1.77	0.49		
HC residual difference						
Monozygotic	35	0.12	0.69	0.12	-1.793	(0.08)
Dizygotic	13	0.57	0.97	0.27		
Absolute value of HC residual difference						
Monozygotic	35	0.48	0.50	0.09	-1.658	(0.10)
Dizygotic	13	0.79	0.78	0.22		

Table 4

Partial correlations with HC, controlling for age and gender

All		Macrocephaly only																						
All twins				Affected twins				Unaffected twins				All twins				Affected twins				Unaffected co-twins				
N	Mean (cm)	R	p	N	Mean (cm)	r	p	N	Mean (cm)	r	p	N	Mean (cm)	r	p	N	Mean (cm)	r	p	N	Mean (cm)	r	p	
FSIQ	379	80.22	-0.158	0.002**	231	67.83	-0.169	0.010*	148	99.56	-0.010	0.902	79	73.01	-0.238	0.033*	48	59.17	-0.233	0.118	28	96.75	-0.002	0.991
NVIQ	383	83.26	-0.143	0.005**	235	72.62	-0.129	0.048*	148	101.15	-0.078	0.352	79	76.19	-0.21	0.060	50	65.48	-0.152	0.302	28	95.32	-0.089	0.666
VIQ	380	78.7	-0.160	0.002**	231	65.30	-0.194	0.003**	149	99.38	0.054	0.516	79	71.68	-0.242	0.029*	48	55.13	-0.249	0.095	29	99.07	0.058	0.773
PPVT	366	87.27	-0.142	0.007**	219	75.63	-0.184	0.007**	147	104.61	0.160	0.055	70	81.74	-0.336	0.004**	46	66.37	-0.282	0.063	28	107.00	-0.223	0.274
VABS	394	79.7	-0.135	0.007**	247	68.10	-0.159	0.013**	147	99.26	0.001	0.993	78	74.09	-0.146	0.197	53	62.53	-0.087	0.545	29	95.20	0.370	0.058*
SRS	336	68.4	0.097	0.076	211	82.04	0.076	0.271	125	45.39	0.083	0.360	54	72.53	0.296	0.027*	37	87.03	0.241	0.163	21	47.00	0.188	0.441

FSIQ full scale IQ, NVIQ nonverbal IQ, VIQ verbal IQ, PPVT peabody picture vocabulary test, VABS vineland adaptive behavior scales, SRS social responsiveness scale

* $p<0.05$,

** $p<0.01$

Table 5

Macrocephaly concordance in twin pairs

<u>All twin pairs</u>		<u>ASD concordant pairs</u>			<u>ASD discordant pairs</u>		
N	One twin with macrocephaly	Both twins with macrocephaly	p	N	One twin with macrocephaly	Both twins with macrocephaly	p
All	202	41	21	53	6	8	13
MZ	56	6	7	35	4	5	2
DZ	146	35	14	18	2	3	11
MZM	48	5	7	32	3	5	2
DZM	50	8	2	9	0	1	1
MZF	8	1	0	3	1	0	0
DZF	14	7	1	4	2	0	1

MZ monozygotic, *DZ* dizygotic, *MZM* monozygotic males, *DZM* dizygotic males, *MZF* monozygotic females, *DZF* dizygotic females

Table 6

Bivariate correlations of HC between twin pairs

All sex concordant twin pairs					Sex concordant, ASD concordant twin pairs					Sex concordant, ASD discordant twin pairs				
N	HC 1	HC 2	Intraclass correlation coefficient r	p	N	HC1	HC2	Intraclass correlation coefficient r	p	N	HC 1 (affected Twins)	HC 2 (unaffected twins)	Interclass correlation coefficient r	p
MZ	56	54.64	54.54	0.818	<0.001***	35	54.61	54.49	0.820	<0.001***	21	54.69	54.69	0.823***
DZ	64	54.23	53.79	0.668	<0.001***	13	54.31	53.00	0.720	0.002**	51	54.21	53.98	0.662***
Heritability				0.30		Heritability			0.2		Heritability			0.32

MZ monzygotic, DZ dizygotic

* $p < 0.05$;

** $p < 0.01$;

*** $p < 0.001$