Published in final edited form as:

Genes Brain Behav. 2010 April ; 9(3): 288–295. doi:10.1111/j.1601-183X.2009.00558.x.

Are There Differences in Brain Morphometry Between Twins and Unrelated Singletons? A Pediatric MRI Study

Sarah J. Ordaz^{1,2}, Rhoshel K. Lenroot^{1,4}, Gregory L. Wallace¹, Liv S. Clasen¹, Jonathan D. Blumenthal¹, J. Eric Schmitt³, and Jay N. Giedd^{1,*}

¹Child Psychiatry Branch, National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, 20892

²Department of Psychology, University of Pittsburgh, Pittsburgh, PA 15213

³Virginia Institute for Psychiatric and Behavioral Genetics and Departments of Psychiatry and Human Genetics, Virginia Commonwealth University, Richmond, VA, 23298

⁴ University of New South Wales and Prince of Wales Medical Research Institute, Sydney, Australia

Abstract

Twins provide a unique capacity to explore relative genetic and environmental contributions to brain development, but results are applicable to non-twin populations only to the extent that twin and singleton brains are alike. A reason to suspect differences is that as a group twins are more likely than singletons to experience adverse prenatal and perinatal events that may affect brain development. We sought to assess whether this increased risk leads to differences in child or adolescent brain anatomy in twins who do not experience behavioral or neurological sequelae during the perinatal period. Brain MRI scans of 185 healthy pediatric twins (mean age=11.0, s.d.=3.6) were compared to scans of 167 age- and sex-matched unrelated singletons on brain structures measured, which included gray and white matter lobar volumes, ventricular volume, and area of the corpus callosum. There were no significant differences between groups for any structure, despite sufficient power for low Type II (i.e. false negative) error. The implications of these results are twofold: (1) within this age range and for these measures, it is appropriate to include healthy twins in studies of typical brain development, and (2) findings regarding heritability of brain structures obtained from twin studies can be generalized to non-twin populations.

Keywords

Twin; Singleton; Brain; Child; Adolescent

Introduction

The study of brain development in twins allows exploration of the relative contributions of genetic and environmental influences to variations in brain structure (e.g., (Baare *et al.*, 2001, Hulshoff Pol *et al.*, 2006, Thompson *et al.*, 2001, Wallace *et al.*, 2006, Wright *et al.*, 2002) (Schmitt *et al.*, 2007) (Schmitt *et al.*, 2008) (Lenroot *et al.*, 2009) (Brun *et al.*, 2009, Peper *et al.*, 2009). The generalizability of these studies rests on the assumption that brain structure in twins is comparable to that of singletons. However, twins are faced with additional challenges during early development. In the intrauterine environment each twin must compete

*Correspondence: Jay N. Giedd, M.D., Bldg 10, Room 4C110, 10 Center Dr., Child Psychiatry Branch, National Institute of Mental Health, Bethesda, MD, 20892, Ph: 301.435.4517 Fax: 301.480.8898 jg@nih.gov.

against the other for limited space and nutritional resources that are fully available to a singleton fetus. Typically developing twins have shorter gestational ages, lower weights given their gestational age (Buckler & Green, 2004, Glinianaia *et al.*, 2000, Liu & Blair, 2002, Powers & Kiely, 1994), and increased risk of perinatal complications (Rao *et al.*, 2004). Studies of preterm, low birth weight singletons have shown that such early developmental disturbances can lead to later reductions in cortical and subcortical brain volumes in childhood (Peterson *et al.*, 2000). It is not known whether the additional stresses of an uncomplicated twin pregnancy have similar adverse effects on the structural development of the brain during childhood and adolescence.

One previous study has been published comparing brain anatomy between twins and their healthy siblings. This study was performed in an adult population and reported a significant difference in cerebral white matter volume that was no longer significant after correcting for twins' smaller intracranial volumes; though importantly, they reported no differences in total brain volume (Hulshoff Pol *et al.*, 2002). However, it is possible that the effects of adverse events occurring in the pre- or perinatal periods gradually diminish, such that differences that are still significant and observable during childhood (Buckler & Green, 2004, Wilson, 1979) are no longer perceptible in adulthood. A longitudinal study of head circumference found that twins had smaller head circumferences that persisted from birth through age 4 (Buckler & Green, 2004), and a longitudinal study found that twins had lower IQs than singletons at age 4, but were no longer different by age 6 (Wilson, 1974). Additionally, a study of physical growth in twins and singletons reported that twins reach parity with singletons in height and weight by late childhood (Wilson, 1979). Thus, pediatric studies using other metrics of brain and cognitive development suggest that by adulthood twins may catch up to singletons, but to date no known studies have examined differences in brain morphometry in pediatric twins and singletons. Determining the comparability of twin and singleton brain development in younger ages is necessary to determine whether results of twin studies in pediatric populations can be generalized to non-twin populations and to establish whether individuals born as twins can be included routinely with singletons in studies of typical brain development.

We therefore compared measures of brain anatomy, including total brain volume, lobar volumes, and area of the corpus callosum, between pediatric twins and unrelated singleton participants. Although scans of siblings of some of the twin subjects have been acquired, we chose to compare to unrelated singletons because of the availability of a much larger sample size and better matching for age and gender which are best suited for the objectives of this study.

Methods

Participants

Healthy child and adolescent twins and singletons were recruited for participation in an ongoing longitudinal pediatric brain MRI study at the Child Psychiatry Branch of the National Institute of Mental Health (NIMH) (Giedd *et al.*, 2009). Singletons were recruited locally and twins locally and nationally. Parents of prospective participants were interviewed by phone and asked to report their child's health, developmental, and educational histories.

Participants were excluded if they had ever taken psychiatric medications, received psychiatric diagnoses, or had any other trauma or condition known to affect gross brain development. Twins diagnosed with twin-to-twin transfusion syndrome during gestation were excluded from the study. Inclusion criteria for both twins and singletons included a minimum gestational age of 29 weeks and a minimum birth weight of 1500 grams. These ranges were chosen to accommodate the typically shorter gestational ages and lower birth weights of twins, yet to exclude neonates whose circumstances are extraordinary and place them at higher risk for

adverse neurodevelopmental outcomes (Nagy, 2003, Peterson *et al.*, 2000, Reiss *et al.*, 2004). Approximately 80% of families responding to advertisements met study criteria.

The NIMH Institutional Review Board approved the protocol. Written informed assent was obtained from all participants under the age of 18 in addition to consent from parents/guardians; individuals 18 years and older provided written informed consent. All subjects were administered the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999) or, for those participants younger than 7 years of age, the Vocabulary, Similarities, Information, Block Design, Matrix Reasoning, Picture Concepts, and Coding subtests of the Wechsler Preschool and Primary Scale of Intelligence, Third Edition (WPPSI-III) (Wechsler, 2002). Using these instruments, Full-Scale IQ as well as Verbal and Performance IQ were calculated. Socioeconomic status (SES) was determined using the Hollingshead SES scale (Hollingshead & Redlich, 2007, Hollingshead & Redlich, 1958). Handedness was obtained using the Physical and Neurological Examination for Soft Signs (PANESS) (Denckla, 1985). Birth data were obtained by parental report. Zygosity of the twins was determined by DNA analysis of buccal cheek swabs using 9-21 unlinked short tandem repeat loci for a minimum certainty of 99% by BRT Laboratories, Inc. (Baltimore, MD).

Scan Acquisition

All images were acquired on the same General Electric 1.5 Tesla Signa Scanner located at the NIH Clinical Center in Bethesda, Maryland. A three-dimensional spoiled gradient recalled echo sequence in the steady state, designed to optimize discrimination between gray matter, white matter and CSF, was used to acquire 124 contiguous 1.5 mm thick slices in the axial plane (TE/TR = 5/24; flip angle = 45 degrees, matrix = 256×192, NEX=1, FOV= 24cm, acquisition time 9.9 min). A Fast Spin Echo/Proton Density weighted imaging sequence was also acquired for clinical evaluation.

Image Analysis

The native MRI scans were registered into standardized stereotaxic space using a linear transformation (Collins *et al.*, 1994) and corrected for non-uniformity artifacts (Sled *et al.*, 1998). The registered and corrected volumes were segmented into white matter, gray matter, and cerebrospinal fluid using a neural net classifier (Zijdenbos *et al.*, 2002). Region of interest analysis was performed by combining tissue classification information with a probabilistic atlas (Collins *et al.*, 1995). The regional volumes which have shown high agreement with conventional hand tracing measures and are included in this analysis are the right-sided, left-sided, and total volumes of the following regions: total cerebrum (which is the sum of the total gray matter and total white matter), total gray matter, total white matter, gray and white matter of the frontal, temporal, parietal, and occipital lobes, as well as the caudate nucleus. A validation study comparing this method with manual segmentation found volumetric differences to be less than 10% and volumetric overlap to be greater than 85% (Collins *et al.*, 1995). An independent validation study of caudate volumes comparing a sample of manually defined caudate volumes from 263 pediatric subjects from this laboratory with automated measures found them to be highly correlated (Spearman's $\rho > .72$, $p < .01$ for left, right, and total caudate volumes). Midsagittal area of the corpus callosum was quantified as per a previously described protocol. (Giedd *et al.*, 1999). Scans were reviewed by a trained rater (JB), and those with gross motion artifact were removed from the sample.

Statistical Analysis

One twin each from 185 twin pairs was randomly selected for analysis using a computerized random selection algorithm. A set of 167 unrelated singletons was selected from the larger singleton study group matched by sex and age at scan (see Table I). Data were screened for outliers, and none were removed for purposes of the analysis. Chi-square tests and analysis of

variance (ANOVA) were used to determine group differences between twins and singletons for right, left, and total sizes of all brain regions.

ANCOVA was used to estimate potential effects of IQ and SES differences on comparison of brain measures between twins and singletons. For IQ, the assumption of homogeneity of regression for ANCOVA was violated in the following regions: total cerebral volume, total gray matter, total white matter, frontal gray matter, temporal white matter, occipital gray matter, occipital white matter, and the mid-sagittal corpus callosum. For these regions, we addressed this violation by following the recommendations of (Tabachnick & Fidell) and (Keppel *et al.*, 2004), transforming IQ into a blocking variable that was then entered as a factor and an interaction factor within a regression framework. To create the blocking variable, individuals were divided into three equally sized IQ groups (low, medium, high). In all other brain regions which did not violate this assumption, ANCOVA was run using a continuous IQ covariate because it has better precision and power. As SES is not a parametric covariate, SES scores were also arbitrarily divided into three similar sized groups and effects on outcome measures predicted using regression. To control for Type I error, a false discovery rate procedure (Benjamini & Hochberg, 1995) was applied to each of the three sets of group comparisons. In order to explore whether group differences in brain volume change with age (given postulations that twin brain growth may catch up during the postnatal period), we tested the interaction of group by age in all of the aforementioned brain regions using a continuous measure of age.

Effect sizes were estimated by calculating $d = (\mu_1 - \mu_2)/\sigma_{\text{diff}}$ (Cohen, 1992), where σ_{diff} is the standard deviation of the difference in means between the two groups. Cohen's conventions for the magnitude of effect size were utilized; effect sizes were defined as 'small' if $d \leq 0.2$, 'medium' if $0.2 < d < 0.8$, and 'large' if $d \geq 0.8$. Given concern for Type II error (i.e. false negative) for this hypothesis, post-hoc criterion power analyses were used to compute the alpha level that would be compatible with a low Type II error ($\beta = 0.05$; $1 - \beta = 0.95$) for a sample of this size and for small effect sizes. Analyses were performed using SPSS 14.0. Sample sizes needed to obtain power were calculated using the G-Power 3.0 software (Faul *et al.*, 2007).

Results

Demographics

Subjects ranged in age from 4.6 to 19.5 years. Means, standard deviations, test statistics, and p-values for all demographic data comparisons are shown in Table I. There were no significant differences between twins and singletons in age or ratio of males to females. The twin sample was composed of a significantly larger percentage of non-Hispanic Caucasian participants (93.0% non-Hispanic Caucasian in twins vs. 77.8% in singletons). There were significant group differences on SES as well as Full Scale IQ, Verbal IQ, and Performance IQ scores; twins were lower SES and had lower IQ scores. Handedness differences were not significant, but twins trended towards having a higher percentage of mixed or left-handed subjects than did singletons.

123 (66.4%) twins were from monozygotic (MZ) pairs, 58 (31.4%) were from same-sex dizygotic (DZ) pairs, and four (2.2%) were from same-sex pairs that had not completed a zygosity test. Of the twins randomly selected for these analyses, 95 (51.4%) were first-born twins, 85 (45.9%) were second-born twins, and the birth order of 5 (2.7%) was unreported. The distribution of males and females did not differ within zygosity subgroups ($\chi^2(2) = .02$, *n.s.*) nor birth order subgroups ($\chi^2(1) = .04$, *n.s.*).

Birth history variables

An examination of birth history including means, standard deviations, F-values, and p-values for comparisons of birth weight and gestational age are shown in Table I. Consistent with previous reports, twins had lower mean birth weights and younger gestational ages compared to singletons.

Volumetric brain measurements

Means, standard deviations, F-values, p-values, and effect sizes for all comparisons of total volumetric brain measurements are shown in Table II and comparisons broken down by laterality are shown in Table III. Following application of the false discovery rate procedure, no significant differences were found for any of the regions analyzed. Cohen's *d* effect size reached a maximum absolute value of 0.074. Significance for the right and left hemispheres in each brain structure followed the same pattern as for each brain structure as a whole. As the lateralized and combined hemisphere results are comparable, for clarity of presentation, analyses are henceforth presented for each given structure as a whole.

Due to significant group differences in IQ and SES, brain volume comparisons were repeated with each of these variables added as covariates. IQ and SES accounted for overlapping variance (Spearman's rho correlation coefficient = -0.324; $p < .001$); separate analyses were therefore run for each of these two covariates as described in the Methods section. An ANCOVA was used to covary for IQ, using a continuous measure of IQ, except in regions in which the assumption of homogeneity of regression for ANCOVA was violated (total cerebral volume, total gray and white matter, frontal gray matter, temporal white matter, occipital gray matter, occipital white matter, and mid-sagittal corpus callosum). In these regions we used a categorical IQ variable as a blocking variable as described in the Methods section. There were no significant differences in brain volume between twins and singletons when covarying IQ as a continuous ($F(1, 338)$, all $ps \geq .298$, see Tables II and III) or categorical variable ($t(335)$, all $ps \geq .197$). A categorical measure was used to covary for SES given that it was not a parametric covariate. Comparisons using this covariate indicated no significant effects of SES on twin-singleton comparisons for any region ($t(331)$, all $ps \geq .083$, see Tables II and III).

To examine whether group differences decreased with age, group by age interactions were tested in a regression framework using a continuous measure of age. Results indicated no significant interactions in any of the regions measured after application of the false discovery rate procedure ($t(348)$, all $ps \geq .022$, see Tables II and III).

Cohen's *d* effect sizes for the uncorrected twin/singleton comparisons were all small in magnitude, ranging from 0.003 to 0.074 for volumetric brain data and from 0.004 to 0.251 for lateralized volumetric brain data (see Tables II and III). Post-hoc criterion power analysis to determine a significance level α , which is compatible with a low Type II error ($\beta = 0.05$), produced a high alpha error probability ($\alpha = 0.410$). Even if this significance criterion was used in order to minimize Type II error, there would be no significant differences in any brain region after correcting for multiple comparisons.

Discussion

We found no significant differences between pediatric twins and singletons for total and lobar gray and white matter volumes, caudate nucleus volumes, ventricular volumes, or area of the corpus callosum. Effect sizes of twin-singleton differences were small (Cohen, 1992), suggesting a high degree of overlap in the distributions of each group's brain volumes regardless of the region assessed. Post-hoc power analyses confirmed that the sample had sufficient power to make a Type II error unlikely. These results support the generalizability of findings obtained

from studies of brain development in twins within this age range to non-twin populations, and the use of twins as subjects in studies of typical brain development.

In the one previous study comparing 112 adult twins and 34 of their healthy non-twin siblings (Hulshoff Pol *et al.*, 2002), the only difference found was in white matter volume, which was reported to be smaller in the adult twins, and no longer significant after controlling for group differences in intracranial volume. Comparison between studies is limited by differences in study design, including adult versus pediatric populations, methods of image acquisition and methods of image analysis. In addition, the present study compared twins to unrelated singletons whereas Hulshoff Pol and colleagues (2002) used twin siblings as a comparison group. Nonetheless, it is possible that white matter differences only emerge after volumetric increases due to myelination during childhood and adolescence, raising the question of whether growth trajectories may be different in twins and singletons.

Our findings indicate that age does not moderate differences in twin and singleton brain volumes between ages 4 and 19. That is, brain volumes do not “catch up” over the course of childhood and adolescence. However, studies comparing growth trajectories of height, weight, and head circumference between twins and singletons have found most pronounced differences at birth that rapidly diminish due to catch-up growth in twins (Wilson, 1979)(Buckler & Green, 2004). Together with this research, our results indicate that catch-up growth, if it occurs at all, is complete by early childhood. Research comparing cognitive development between twins and singletons has produced mixed results. Studies using data acquired from children in the 1940s and 1950s found twins have IQs that are on average five points lower than IQs of singletons (Deary *et al.*, 2005, Drillien, 1961, Record *et al.*, 1970)(Mehrotra & Maxwell, 1949, Santon, 1957). A subsequent study (Wilson, 1974) found an IQ difference among four and five year olds that was not present among six year olds, suggesting differences may be due to a temporary developmental lag in twins. Recent studies of adolescents (Christensen *et al.*, 2006) and adults (Posthuma *et al.*, 2000) found no difference in cognitive performance in twins compared to singletons. Studies of heritability of intelligence have found that IQ becomes increasingly heritable with age, suggesting that cognitive abilities may also have some resilience to early adverse environmental influences (McClearn *et al.*, 1997, Plomin & Kosslyn, 2001, Plomin *et al.*, 1994). Within our sample, IQ was approximately five points lower in twins than in singletons. However, interpretation of this finding is complicated by the significantly lower SES of twins in our sample, likely due to ascertainment bias.

The present study possesses several limitations. The twins included in this study were not chosen to be representative of the twin population as a whole, but rather representative of twins who are likely to meet typical screening criteria as subjects for studies of normal brain development. Both twins and singletons with a history of severe adverse birth events, atypically short gestational age (less than 29 weeks), or very low birth weight (less than 1500g) were excluded from the present study. These risk factors have been shown to affect brain development into childhood and adolescence and are more likely to occur in twins (Parker *et al.*, 2008, Peterson *et al.*, 2000). Twins (and group-matched singletons) from our study had higher than average IQ scores, which may limit generalizability to the twin and singleton populations. It should be noted that a goal of this study was to explore whether results from twin studies of brain morphometry can be related to comparable research conducted in singletons. As many published studies of brain development in twins (Lenroot *et al.*, 2009) (Schmitt *et al.*, 2008, Schmitt *et al.*, 2007)(Wallace *et al.*, 2006) and singletons (Reiss *et al.*, 1996)(Shaw *et al.*, 2006)(Lenroot *et al.*, 2007)(Paus *et al.*, 1999)(Lu, 2009) have used samples with similarly high IQ scores (when these scores are obtained and reported), we believe that the participants in this study are representative of individuals who volunteer and meet screening criteria for imaging studies in general. It should be noted that the youngest participants in the current study were four years old, and that results should not be taken as indicative of findings

in infants and very young children as previously mentioned. Furthermore, the current study is cross-sectional; future studies using longitudinal data will allow determination of whether developmental trajectories differ between twins and singletons. Finally, our conclusions can only be held as valid for the structures reported. Studies using higher-resolution techniques such as voxel-based measurements of cortical features may detect more subtle twin-singleton differences.

In summary, this study demonstrates that brain lobar volumes, ventricular size, and area of the corpus callosum are not different between twins and singletons during childhood and adolescence. This supports the utility of brain morphometric data obtained from twins during childhood and adolescence in studies of healthy brain development, and the external validity of large-scale twin studies in exploring genetic and environmental sources of variation in brain development.

Acknowledgments

This research reported in this paper was supported by the Intramural Research Program of the NIH, National Institute of Mental Health; NIH grants MH-20030 and MH-65322.

We thank the participants and their families for their time. In addition, we thank Elizabeth Molloy, Michael Rosenthal, Blythe Rose, and Kristin Taylor for their assistance with data collection.

References

- Baare WF, Hulshoff Pol HE, Boomsma DI, Posthuma D, de Geus EJ, Schnack HG, van Haren NE, van Oel CJ, Kahn RS. Quantitative genetic modeling of variation in human brain morphology. *Cereb Cortex* 2001;11:816–824. [PubMed: 11532887]
- Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)* 1995;57:289–300.
- Brun CC, Lepore N, Pennec X, Lee AD, Barysheva M, Madsen SK, Avedissian C, Chou YY, de Zubicaray GI, McMahon KL, Wright MJ, Toga AW, Thompson PM. Mapping the regional influence of genetics on brain structure variability--a tensor-based morphometry study. *Neuroimage* 2009;48:37–49. [PubMed: 19446645]
- Buckler JM, Green M. A comparison of the early growth of twins and singletons. *Ann Hum Biol* 2004;31:311–332. [PubMed: 15204347]
- Christensen K, Petersen I, Skytthe A, Herskind AM, McGue M, Bingley P. Comparison of academic performance of twins and singletons in adolescence: follow-up study. *BMJ* 2006;333:1095. [PubMed: 17012267]
- Cohen J. A power primer. *Psychol Bull* 1992;112:155–159. [PubMed: 19565683]
- Collins DL, Holmes CJ, Peters TM, Evans AC. Automatic 3-D model-based neuroanatomical segmentation. *Hum Brain Mapp* 1995;3:190–208.
- Collins DL, Neelin P, Peters TM, Evans AC. Automatic 3D intersubject registration of MR volumetric data in standardized Talairach space. *J Comput Assist Tomogr* 1994;18:192–205. [PubMed: 8126267]
- Deary IJ, Pattie A, Wilson V, Whalley LJ. The cognitive cost of being a twin: Two whole-population surveys. *Twin Research and Human Genetics* 2005;8:376–383. [PubMed: 16176723]
- Denckla MB. Revised physical and neurological examination for subtle signs. *Psychopharmacol Bull* 1985;21:773–800. [PubMed: 4089106]
- Drillien CM. The incidence of mental and physical handicaps in school-age children of very low birth weight. *Pediatrics* 1961;27:452–464. [PubMed: 13724419]
- Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods* 2007;39:175–191. [PubMed: 17695343]
- Giedd JN, Blumenthal J, Jeffries NO, Rajapakse JC, Vaituzis AC, Liu H, Berry YC, Tobin M, Nelson J, Castellanos FX. Development of the human corpus callosum during childhood and adolescence: A

- longitudinal MRI study. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 1999;23:571–588. [PubMed: 10390717]
- Giedd JN, Lalonde FM, Celano MJ, White SL, Wallace GL, Lee NR, Lenroot RK. Anatomical brain magnetic resonance imaging of typically developing children and adolescents. *J Am Acad Child Adolesc Psychiatry* 2009;48:465–470. [PubMed: 19395901]
- Glinianaia SV, Skjaerven R, Magnus P. Birthweight percentiles by gestational age in multiple births. A population-based study of Norwegian twins and triplets. *Acta Obstet Gynecol Scand* 2000;79:450–458. [PubMed: 10857868]
- Hollingshead AB, Redlich FC. Social class and mental illness: a community study. 1958. *Am J Public Health* 2007;97:1756–1757. [PubMed: 17895405]
- Hollingshead, AdB; Redlich, FC. Social class and mental illness; a community study. Wiley; New York: 1958.
- Hulshoff Pol HE, Posthuma D, Baare WF, De Geus EJ, Schnack HG, van Haren NE, van Oel CJ, Kahn RS, Boomsma DI. Twin-singleton differences in brain structure using structural equation modelling. *Brain* 2002;125:384–390. [PubMed: 11844738]
- Hulshoff Pol HE, Schnack HG, Posthuma D, Mandl RC, Baare WF, van Oel C, van Haren NE, Collins DL, Evans AC, Amunts K, Burgel U, Zilles K, de Geus E, Boomsma DI, Kahn RS. Genetic contributions to human brain morphology and intelligence. *J Neurosci* 2006;26:10235–10242. [PubMed: 17021179]
- Keppel KG, Percy JN, Klein RJ. Measuring progress in Healthy People 2010. *Healthy People 2010 Stat Notes* 2004;1–16. [PubMed: 15446274]
- Lenroot RK, Gogtay N, Greenstein DK, Wells EM, Wallace GL, Clasen LS, Blumenthal JD, Lerch J, Zijdenbos AP, Evans AC, Thompson PM, Giedd JN. Sexual dimorphism of brain developmental trajectories during childhood and adolescence. *Neuroimage* 2007;36:1065–1073. [PubMed: 17513132]
- Lenroot RK, Schmitt JE, Ordaz SJ, Wallace GL, Neale MC, Lerch JP, Kendler KS, Evans AC, Giedd JN. Differences in genetic and environmental influences on the human cerebral cortex associated with development during childhood and adolescence. *Hum Brain Mapp* 2009;30:163–174. [PubMed: 18041741]
- Liu YC, Blair EM. Predicted birthweight for singletons and twins. *Twin Res* 2002;5:529–537. [PubMed: 12573184]
- McClearn GE, Johansson B, Berg S, Pedersen NL, Ahern F, Petrill SA, Plomin R. Substantial genetic influence on cognitive abilities in twins 80 or more years old. *Science* 1997;276:1560–1563. [PubMed: 9171059]
- Mehrotra SN, Maxwell J. The intelligence of twins. A comparative study of eleven-year-old twins. *Population Studies* 1949;3:295.
- Nagy PS. Spontaneous rupture of the uterus caused by placenta percreta at 28 weeks of twin pregnancy. *Eur J Obstet Gynecol Reprod Biol* 2003;111:207–209. [PubMed: 14597253]
- Parker J, Mitchell A, Kalpakidou A, Walshe M, Jung HY, Nosarti C, Santosh P, Rifkin L, Wyatt J, Murray RM, Allin M. Cerebellar growth and behavioural & neuropsychological outcome in preterm adolescents. *Brain* 2008;131:1344–1351. [PubMed: 18372312]
- Paus T, Zijdenbos A, Worsley K, Collins DL, Blumenthal J, Giedd JN, Rapoport JL, Evans AC. Structural maturation of neural pathways in children and adolescents: in vivo study. *Science* 1999;283:1908–1911. [PubMed: 10082463]
- Peper JS, Schnack HG, Brouwer RM, Van Baal GC, Pjetri E, Szekely E, van Leeuwen M, van den Berg SM, Collins DL, Evans AC, Boomsma DI, Kahn RS, Hulshoff Pol HE. Heritability of regional and global brain structure at the onset of puberty: a magnetic resonance imaging study in 9-year-old twin pairs. *Hum Brain Mapp* 2009;30:2184–2196. [PubMed: 19294640]
- Peterson BS, Vohr B, Staib LH, Cannistraci CJ, Dolberg A, Schneider KC, Katz KH, Westerveld M, Sparrow S, Anderson AW, Duncan CC, Makuch RW, Gore JC, Ment LR. Regional brain volume abnormalities and long-term cognitive outcome in preterm infants. *JAMA* 2000;284:1939–1947. [PubMed: 11035890]
- Plomin R, Kosslyn SM. Genes, brain and cognition. *Nat Neurosci* 2001;4:1153–1154. [PubMed: 11723454]

- Plomin R, Pedersen NL, Lichtenstein P, McClearn GE. Variability and stability in cognitive abilities are largely genetic later in life. *Behav Genet* 1994;24:207–215. [PubMed: 7945151]
- Posthuma D, De Geus EJ, Bleichrodt N, Boomsma DI. Twin-singleton differences in intelligence? *Twin Res* 2000;3:83–87. [PubMed: 10918620]
- Powers WF, Kiely JL. The risks confronting twins: a national perspective. *Am J Obstet Gynecol* 1994;170:456–461. [PubMed: 8116697]
- Rao A, Sairam S, Shehata H. Obstetric complications of twin pregnancies. *Best Pract Res Clin Obstet Gynaecol* 2004;18:557–576. [PubMed: 15279817]
- Record RG, Mckeown T, Edwards JH. An Investigation of Difference in Measured Intelligence between Twins and Single Births. *Annals of Human Genetics* 1970;34:11–&. [PubMed: 5529232]
- Reiss AL, Abrams MT, Singer HS, Ross JL, Denckla MB. Brain development, gender and IQ in children. A volumetric imaging study. *Brain* 1996;119:1763–1774. [PubMed: 8931596]
- Reiss AL, Kesler SR, Vohr B, Duncan CC, Katz KH, Pajot S, Schneider KC, Makuch RW, Ment LR. Sex differences in cerebral volumes of 8-year-olds born preterm. *The Journal of Pediatrics* 2004;145:242–249. [PubMed: 15289777]
- Santon F. The relative numbers and abilities of some ten-year-old twins. *Journal of the Royal Statistical Society. Series B (Methodological)* 1957;120:440.
- Schmitt JE, Lenroot RK, Wallace GL, Ordaz S, Taylor KN, Kabani N, Greenstein D, Lerch JP, Kendler KS, Neale MC, Giedd JN. Identification of Genetically Mediated Cortical Networks: A Multivariate Study of Pediatric Twins and Siblings. *Cereb Cortex*. 2008 E Pub ahead of print.
- Schmitt JE, Wallace GL, Rosenthal MA, Molloy EA, Ordaz S, Lenroot R, Clasen LS, Blumenthal JD, Kendler KS, Neale MC, Giedd JN. A multivariate analysis of neuroanatomic relationships in a genetically informative pediatric sample. *Neuroimage* 2007;35:70–82. [PubMed: 17208460]
- Shaw P, Greenstein D, Lerch J, Clasen L, Lenroot R, Gogtay N, Evans A, Rapoport J, Giedd J. Intellectual ability and cortical development in children and adolescents. *Nature* 2006;440:676–679. [PubMed: 16572172]
- Sled JG, Zijdenbos AP, Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans Med Imaging* 1998;17:87–97. [PubMed: 9617910]
- Tabachnick, BG.; Fidell, LS. *Computer-assisted research design and analysis*. Allyn and Bacon; Needham heights, MA: p. 130-132.
- Thompson PM, Cannon TD, Narr KL, van Erp T, Poutanen VP, Huttunen M, Lonnqvist J, Standertskjold-Nordenstam CG, Kaprio J, Khaledy M, Dail R, Zoumalan CI, Toga AW. Genetic influences on brain structure. *Nat Neurosci* 2001;4:1253–1258. [PubMed: 11694885]
- Wallace GL, Schmitt JE, Lenroot R, Viding E, Ordaz S, Rosenthal MA, Molloy EA, Clasen LS, Kendler KS, Neale MC, Giedd JN. A pediatric twin study of brain morphometry. *Journal of Child Psychology and Psychiatry* 2006;47:987–993. [PubMed: 17073977]
- Wechsler, D. *Wechsler Abbreviated Scale of Intelligence*. The Psychological Corporation; San Antonio, Texas: 1999.
- Wechsler, D. *Wechsler preschool and primary scale of intelligence*. 3rd. Psychological Corp. :Harcourt Brace Jovanovich; San Antonio: 2002.
- Wilson RS. Growth standards for twins from birth to four years. *Ann Hum Biol* 1974;1:175–188. [PubMed: 4472129]
- Wilson RS. Twin growth: initial deficit, recovery, and trends in concordance from birth to nine years. *Ann Hum Biol* 1979;6:205–220. [PubMed: 573983]
- Wright IC, Sham P, Murray RM, Weinberger DR, Bullmore ET. Genetic contributions to regional variability in human brain structure: methods and preliminary results. *Neuroimage* 2002;17:256–271. [PubMed: 12482082]
- Zijdenbos AP, Forghani R, Evans AC. Automatic “pipeline” analysis of 3-D MRI data for clinical trials: application to multiple sclerosis. *IEEE Trans Med Imaging* 2002;21:1280–1291. [PubMed: 12585710]

Table 1

Demographic Data

Variable	Singleton mean (s.d.)	Twin mean (s.d.)	Twin/Singleton comparison Test statistic	p
Age	11.0 (3.6)	10.9 (3.6)	$F(1, 350) = 0.13$	0.716
Sex (% male)	52.7%	53.5%	$\chi^2(1, N = 352) = 0.02$	0.878
Race/Ethnicity (% non-Hispanic Caucasian)	77.8%	93.0%	$\chi^2(1, N = 352) = 16.48$	0.000**
Handedness (% right-handed)	91.6%	84.2%	$\chi^2(2, N = 350) = 5.77$	0.056
SES [†]	37.7 (19.9)	43.3 (17.5)	$F(1, 335) = 7.46$	0.007**
Full Scale IQ	115.8 (13.4)	110.3 (12.5)	$F(1, 339) = 15.45$	0.000**
Verbal IQ	114.5 (12.6)	109.7 (13.2)	$F(1, 265) = 7.66$	0.006**
Performance IQ	113.7 (12.2)	108.7 (13.4)	$F(1, 266) = 8.63$	0.004**
Birth Weight (ounces)	122.8 (17.6)	94.0 (18.6)	$F(1, 286) = 161.62$	0.000**
Gestational Age (weeks)	39.7 (1.8)	37.0 (2.5)	$F(1, 252) = 33.69$	0.000**

[†] Higher values for the SES variable indicate lower socioeconomic status

Table II

Volumetric Brain Data

Brain Region	Singleton mean (s.d.) in cubic cm	Twin mean (s.d.) in cubic cm	Twin/Singleton comparison [†]		Twin/Singleton comparison controlling for IQ			Twin/Singleton comparison controlling for SES			Group × Age interaction		
			F(1, 350)	p	Cohen's d [‡]	Test statistic	p	Effect size*	t(331)	p	p ²	t(348)	p
Total cerebral volume	1162.65 (121.2)	1164.57 (109.6)	0.02	0.88	0.003	<i>t</i> (335)=0.87	0.39	<i>pr</i> ² =0.002	0.24	0.81	0.000	1.25	0.21
Total gray matter	738.65 (77.81)	738.41 (66.66)	0.00	0.97	-0.039	<i>t</i> (335)=1.00	0.32	<i>pr</i> ² =0.003	0.06	0.95	0.000	1.02	0.31
Total white matter	424.00 (57.13)	426.17 (54.62)	0.13	0.72	-0.017	<i>t</i> (335)=0.61	0.55	<i>pr</i> ² =0.001	0.41	0.69	0.000	1.27	0.21
Frontal gray matter	234.26 (24.96)	233.28 (22.02)	0.15	0.70	0.042	<i>t</i> (335)=0.16	0.88	<i>pr</i> ² =0.000	-0.32	0.75	0.000	0.91	0.37
Frontal white matter	162.98 (22.60)	163.63 (21.87)	0.07	0.78	-0.029	<i>F</i> (1,338)=0.68	0.41	<i>η</i> ² =0.002	0.40	0.69	0.000	0.97	0.33
Parietal gray matter	125.85 (15.63)	125.00 (13.35)	0.30	0.59	0.058	<i>F</i> (1,338)=0.01	0.91	<i>η</i> ² =0.000	-1.74	0.08	0.009	1.39	0.17
Parietal white matter	82.59 (11.87)	82.66 (11.61)	0.00	0.96	-0.006	<i>F</i> (1,338)=0.65	0.42	<i>η</i> ² =0.002	0.07	0.95	0.000	0.83	0.41
Temporal gray matter	189.51 (20.20)	189.83 (18.15)	0.02	0.88	-0.017	<i>F</i> (1,338)=1.10	0.30	<i>η</i> ² =0.003	0.43	0.67	0.001	0.92	0.36
Temporal white matter	91.43 (13.08)	91.69 (12.16)	0.04	0.85	-0.021	<i>t</i> (335)=0.97	0.33	<i>pr</i> ² =0.003	0.20	0.84	0.000	0.73	0.47
Occipital gray matter	65.12 (10.18)	64.80 (9.09)	0.01	0.76	0.033	<i>t</i> (335)=0.41	0.68	<i>pr</i> ² =0.000	0.33	0.74	0.000	1.79	0.08
Occipital white matter	40.36 (6.73)	40.85 (6.86)	0.45	0.51	-0.072	<i>t</i> (335)=1.29	0.20	<i>pr</i> ² =0.005	1.12	0.26	0.004	1.07	0.29
Lateral Ventricles	10.23 (6.13)	10.68 (6.04)	0.49	0.49	-0.074	<i>F</i> (1,338)=0.29	0.75	<i>η</i> ² =0.000	0.63	0.53	0.001	-2.30	0.02§
Caudate Nucleus	9.87 (0.99)	9.86 (1.05)	0.00	0.98	0.010	<i>F</i> (1,338)=0.28	0.60	<i>η</i> ² =0.001	-1.64	0.10	0.008	0.98	0.33
Mid-Sagittal Corpus Callosum (area in cm ²)	527.01 (78.1)	530.86 (73.16)	0.23	0.63	-0.051	<i>t</i> (335)=1.01	0.31	<i>pr</i> ² =0.003	0.33	0.75	0.000	-0.73	0.94

[†] Statistics reflect twin/singleton comparisons that are *not* corrected for IQ or SES

[‡] Negative value indicates smaller twin volume

[§] n.s. after false discovery rate correction*Effect sizes are reported as partial eta squared (η^2) for regions in which a continuous covariate was used and as a partial correlation (p^2) for regions in which a categorical covariate was used

Table III
Lateralized Volumetric Brain Data

Brain Region	Twin Mean (s.d.) in cubic cm	Singleton Mean (s.d.) in cubic cm	Twin/Singleton Comparison [†]		Twin/Singleton comparison controlling for IQ		Twin/Singleton comparison controlling for SES		Group × Age interaction				
			F(1, 350)	p	Cohen's d [‡]	Test statistic	p	Effect size *	t(331)	p	pr ²	t(348)	p
Frontal gray matter – left	117.938 (12.498)	117.194 (11.087)	0.35	0.56	0.063	F(1,338)=0.00	0.96	η ² =0.000	-0.50	0.62	0.005	0.87	0.38
Frontal gray matter – right	116.325 (12.554)	116.083 (11.154)	0.04	0.85	0.022	F(1,338)=0.22	0.64	η ² =0.001	-0.13	0.90	0.015	0.93	0.35
Frontal white matter – left	81.625 (11.324)	81.359 (10.981)	0.05	0.82	0.023	F(1,338)=0.13	0.72	η ² =0.000	-0.09	0.93	0.000	0.91	0.36
Frontal white matter – right	81.355 (11.366)	82.271 (10.971)	0.59	0.44	-0.082	F(1,338)=1.65	0.20	η ² =0.005	0.89	0.37	0.002	0.67	0.50
Parietal gray matter – left	62.369 (7.96)	61.696 (6.762)	0.73	0.40	0.091	F(1,338)=0.02	0.90	η ² =0.000	-1.03	0.31	0.003	0.99	0.32
Parietal gray matter – right	63.478 (7.97)	63.307 (6.933)	0.05	0.83	0.023	F(1,338)=0.13	0.72	η ² =0.000	-0.66	0.51	0.001	1.73	0.09
Parietal white matter – left	40.705 (5.954)	40.857 (5.829)	0.06	0.81	-0.026	t(335)=1.02	0.31	p ² =0.003	0.28	0.78	0.000	0.91	0.36
Parietal white matter – right	41.889 (6.052)	41.806 (5.970)	0.02	0.90	0.014	F(1,338)=0.26	0.61	η ² =0.001	-0.15	0.88	0.000	0.73	0.47
Temporal gray matter – left	93.202 (9.925)	93.161 (9.010)	0.12	0.73	0.004	t(335)=0.79	0.43	p ² =0.002	0.32	0.75	0.000	1.07	0.29
Temporal gray matter – right	96.306 (10.449)	96.669 (9.368)	0.00	0.97	-0.037	F(1,338)=1.56	0.21	η ² =0.005	0.52	0.61	0.001	0.76	0.45
Temporal white matter – left	45.141 (6.583)	44.931 (6.009)	0.10	0.75	0.033	t(335)=0.47	0.64	p ² =0.001	-0.25	0.80	0.000	0.69	0.49
Temporal white matter – right	46.293 (6.624)	46.761 (6.300)	0.46	0.50	-0.072	t(335)=1.43	0.15	p ² =0.006	0.63	0.53	0.001	0.76	0.45
Occipital gray matter – left	33.899 (5.272)	33.621 (4.979)	0.26	0.61	0.054	F(1,338)=.12	0.73	η ² =0.000	0.12	0.90	0.000	1.49	0.14
Occipital gray matter – right	31.224 (5.217)	31.182 (4.590)	0.01	0.94	0.009	t(335)=0.56	0.57	p ² =0.001	0.52	0.61	0.001	1.95	0.05
Occipital white matter – left	20.066 (3.424)	20.946 (3.594)	5.50	0.02 [§]	-0.251	t(335)=2.88	0.00 [§]	p ² =0.024	2.77	0.01 [§]	0.023	1.19	0.23
Occipital white matter – right	20.295 (3.535)	19.900 (3.601)	1.08	0.30	0.111	t(335)=-0.39	0.70	p ² =0.000	-0.59	0.56	0.001	0.85	0.40

Brain Region	Twin Mean (s.d.) in cubic cm	Singleton Mean (s.d.) in cubic cm	Twin/Singleton Comparison [†]		Twin/Singleton comparison controlling for IQ		Twin/Singleton comparison controlling for SES		Group × Age interaction			
			F(1, 350)	p	Cohen's d [‡]	Test statistic	p	Effect size *	t(331)	p	t(348)	p
Lateral Ventricles – left	5.265 (3.306)	5.469 (3.308)	0.33	0.56	-0.062	F(1,338)=0.06	0.80	η ² =0.000	0.41	0.69	-2.59	0.01\$
Lateral Ventricles – right	4.96 (3.127)	5.208 (3.083)	0.56	0.45	-0.080	F(1,338)=0.26	0.61	η ² =0.001	0.80	0.42	-1.73	0.09
Caudate Nucleus – left	4.971 (0.516)	5.008 (0.539)	0.45	0.50	-0.070	F(1,338)=1.59	0.21	η ² =0.005	0.70	0.48	1.12	0.26
Caudate Nucleus – right	4.896 (0.495)	4.856 (0.532)	0.54	0.46	0.078	F(1,338)=0.06	0.81	η ² =0.000	-0.52	0.60	0.81	0.42

[†] Statistics reflect twin/singleton comparisons that are *not* corrected for IQ or SES

[‡] Negative value indicates smaller twin volume

[§] n.s. after false discovery rate correction

^{*} Effect sizes are reported as partial eta squared (η^2) for regions in which a continuous covariate was used and as a partial correlation (ρ^2) for regions in which a categorical covariate was used