

Discordance of Prenatal and Neonatal Brain Development in Twins

Niyati Mukherjee¹, Chaeryon Kang¹, Honor Wolfe², Barbara Hertzberg³, Keith Smith⁴, Weili Lin⁴, Guido Gerig⁵, Robert Hamer¹, John H. Gilmore¹

¹Department of Psychiatry, University of North Carolina School of Medicine, Chapel Hill, NC

²Department of Obstetrics and Gynecology, University of North Carolina School of Medicine, Chapel Hill, NC

³Department of Radiology, Duke University Medical Center, Durham, NC

⁴Department of Radiology, University of North Carolina School of Medicine, Chapel Hill, NC

⁵Scientific Computing and Imaging, University of Utah, Salt Lake City, UT

Corresponding Author:

John H. Gilmore, MD

Department of Psychiatry, CB# 7160

University of North Carolina at Chapel Hill

Chapel Hill, NC 27599-7160

Phone: 919-966-6971

Fax: 919-966-8994

jgilmore@med.unc.edu

Word count : *FILL IN ONCE COMPLETE*

2 figures, 5 tables

Abstract

Background: Discordance of birth weight has been observed in twin pairs, though little is known about prenatal and early neonatal discordance of head and brain size, and the role that zygosity and chorionicity play in discordances of early brain development in twins.

Aims: To compare prenatal and neonatal discordances of head size in monozygotic – monozygotic (MZ-MC), monozygotic-dichorionic (MZ-DC), and same-sex dizygotic-dichorionic twin pairs (DZ-DC).

Study Design: Subjects prospectively had ultrasounds at 22 and 32 weeks gestational age, and magnetic resonance imaging (MRI) of the brain MRI after birth.

Subjects: 88 twin pairs recruited from two university hospital prenatal diagnostic clinics; 22 MZ-MC, 17 MZ-DC, and 49 same sex DZ-DC pairs.

Outcome measures: Discordance of head circumference (HC) and weight at 22 weeks, 32 weeks and birth, as well as intracranial volume (ICV) on neonatal MRI.

Results: There were no group differences in discordance of head circumference and weight on the 22 or 32 week ultrasounds, or at birth. MZ-MC twins tended to have numerically greater discordances of HC and weight. There was a significant group difference in ICV on neonatal MRI (ANOVA, $p = 0.0143$), with DZ-DC twins having significantly greater discordance than MZ-MC ($p = 0.028$) or MZ-DC ($p = 0.0131$) twins.

Conclusions: This study indicates that zygosity and chorionicity do not contribute to significant discordances of head size in late prenatal development. DZ twins do have significantly greater discordances of ICV on neonatal MRI, suggesting a relatively greater genetic influence on brain growth in the first weeks after birth.

Introduction

Twin studies compare phenotypic concordance in monozygotic (MZ) and dizygotic (DZ) twins to estimate the heritability of and environmental contributions to development of a trait or disease. The twin study methodology has been applied to many neuropsychiatric and neurodevelopmental disorders, yielding heritability estimates of greater than 90% for autism (Freitag 2007), 70-88% for epilepsy (Kjeldsen et al., 2001), 81% for schizophrenia (Sullivan et al., 2003), and 70-80% for ADHD (Martin et al., 2002).

Twin studies are based on the “equal-environment assumption” that twins have identical prenatal environments (Kendler et al., 1993). However, adverse prenatal environments experienced by MZ twins may predispose them to disease and violate this assumption (Phillips 1993). One-third of MZ twin pregnancies are dichorionic (DC) and two-thirds are monozygotic (MC) (Machin 1995); DZ twins are DC. Differences in placental status and thus in intrauterine environment may result in discordant growth.

Estimates of the incidence of discordant growth in twin pregnancies range from 10 to 30% (Kingdom et al., 2005; Bagchi and Salihu, 2006). Discordance of birth weight has been associated with increased risk of neurologic morbidity in preterm twins (Adegbite et al., 2004), increased risk of morbidity and mortality in small for gestational age twins (Blickstein and Keith, 2004; Yinon et al., 2005) and in twins born at term (Hack et al., 2008). Overall, birth weight discordance has been shown to be greater in MC than DC twins (Corey et al., 1979; Dube et al., 2002; Gonzalez-Quintero et al., 2003; Race et al., 2006; Hack et al., 2008; but see Chauhan et al., 2004; Blickstein et al., 2006).

There have been a few studies that indicate that twins also have divergent brain growth. Prenatal biparietal diameter is discordant in MZ and DZ twins; this discordance becomes greater

at later gestational ages (Persson and Grennert, 1979). We have previously shown in a retrospective study that MZ twins have discordances of head circumference and biparietal diameter on ultrasound in the second trimester that are similar to those observed in DZ twins (Gilmore et al., 1996). This pattern of MZ twins having similar discordances as DZ twins was also seen in subsequent studies of prenatal biparietal diameter (Charlemaïne et al., 1997) and head circumference (Charlemaïne et al., 2000).

The aim of this study is to better characterize the effects of zygosity and chorionicity on discordance of prenatal and neonatal measures of brain size using prenatal ultrasound and neonatal MRI. Additional aims include the examination of: 1) the relationship of discordance of discordance of brain structure and weight; 2) the development of discordance over time; and 3) prenatal challenges and perinatal morbidities as predictors and outcomes of growth discordance.

Methods

Subjects

This study was approved by the Institutional Review Board of the University of North Carolina (UNC) School of Medicine and Duke University Medical Center (DUMC). Mothers with same-sex twin pregnancies were recruited from the outpatient OB-GYN clinics at UNC Hospitals from 2004 onwards and from the OB-GYN clinics at DUMC from 2004 to 2006. Exclusion criteria included maternal HIV infection, major congenital abnormality on fetal ultrasound, and chromosomal abnormalities of fetuses. Fetal ultrasounds were performed in the second and/or third trimester, depending on gestational age at the time of recruitment, and MRI scans were performed at term, approximately 40 weeks gestational age. For zygosity testing, PCR-STR analysis of 14 loci was performed on DNA isolated from buccal swab cell collection (BRT Laboratories, Baltimore, MD). Chorionicity of twin pairs was determined by placental pathology (N=63) or ultrasound if placental pathology was not performed or unavailable (N=25).

Birth weight, length and head circumference were obtained from delivery records. Pregnancy and delivery records were reviewed for the presence of pregnancy, birth and neonatal complications.

Image Acquisition

Mothers who were recruited in time for ultrasounds at 20-24 and/or 30-34 weeks gestation age received study ultrasounds in which head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC), and femur length (FL) were measured. Estimated fetal weight (EFW) was calculated by the Hadlock A formula (Hadlock et al., 1984). Ultrasounds were done on an ATL Philips Ultramark HDI 5000 (Philips, Amsterdam, Netherlands) or GE Voluson Expert (General Electric, Fairfield, CT) and were performed at UNC or DUMC by one

of two study sonographers at each site. Ultrasounds were clinically reviewed by a maternal-fetal physician (HMW) at UNC or a fetal ultrasound radiology specialist (BH) at DUMC.

MRI scans were done on a Siemens 3T head-only scanner (Allegra, Siemens Medical System, Erlangen, Germany). Neonates were scanned unsedated; subjects were fed before scanning, swaddled, given ear protection, and held in place with a vacuum-fixation device for the head. A nurse was present during all scans, and heart rate and oxygen saturation were monitored with a pulse oximeter. T1-weighted structural pulse sequences were T1w structural pulse sequences were either a 3D magnetization prepared rapid gradient echo (MP-RAGE TR/TI/TE/Flip Angle 1820/400/4.38ms/7°) or a 3D spoiled gradient (FLASH TR/TE/Flip Angle 15/7msec/25°). Proton density and T2 weighted images were obtained with a turbo spin echo sequence (TSE TR/TE1/TE2/Flip Angle 6200/20/119ms/150°). Spatial resolution was 1 x 1 x 1mm voxel for T1 weighted images, 1.25 x 1.25 x 1.5mm voxel with 0.5 mm interslice gap for proton density/T2 weighted images. MRIs were clinically reviewed by a neuroradiologist (JKS).

Image Analysis

Brain tissue was automatically classified as gray matter, non-myelinated white matter, myelinated white matter and cerebrospinal fluid using an atlas-moderated iterative expectation maximization segmentation algorithm as previously described (Prastawa et al., 2005; Gilmore et al., 2007). Intracranial volume (ICV) was the sum of the automatic full brain segmentation results for gray, white and CSF (ventricles and subarachnoid space) volumes.

Statistical Analysis

Intra-twin pair discordance of measures of brain and body size was represented as both absolute difference and as relative discordance (defined as |intrapair difference|/measurement in larger twin). Relative discordance was included to correct for baseline differences in size at the

time of scanning. One-way analysis of variance (ANOVA) was used to detect differences in intrapair discordance of biometrics from the second and third trimester ultrasounds, birth, and neonatal MRI between the MZ-MC, MZ-DC, and DZ groups. Pearson's correlation coefficients were calculated to examine the relationship between intrapair discordance of weight and intrapair discordance of other biometrics. Linear regression on weight and head circumference discordance over time was used to look for differences in development of discordance between the three zygosity/chorionity groups. For analysis of incidence of prenatal and postnatal complications in the three groups, Fisher's exact test was used for categorical variables and one-way ANOVA for continuous variables. Additional analysis of prenatal and postnatal complications was performed by the same method with twin pairs divided into birth weight discordance groups based on relative birth weight discordance $<15\%$ or $\geq 15\%$. All statistical hypothesis tests were conducted at a significance level of 0.05.

Results

The final study sample consisted of 88 twin pairs with successful T1 scans, including 22 MZ-MC, 17 MZ-DC, and 49 DZ twin pairs. Within this sample, abnormalities noted on review of ultrasounds included polyhydramnios (5 subjects), oligohydramnios (6 subjects), twin-twin transfusion syndrome (1 subject), left ventricular echogenic focus (3 subjects), choroid plexus cyst (2 subjects), dangling/droopy choroid (1 subject), mild pelviectasis (8 subjects), hydronephrosis (2 subjects), and multicystic kidney (1 subject). Abnormalities noted on review of MRIs included mild ventriculomegaly (4 subjects), small subdural hemorrhages (4 subjects), calcifications (3 subjects), prominent CSF spaces (2 subjects), periventricular leukomalacia (1 subject), and dilated periventricular spaces (1 subject). None of these subjects were excluded from this analysis. There were no significant differences in maternal age or ethnicity, gender, gestational age at birth, or gestational age at second trimester ultrasound, third trimester ultrasound, or neonatal MRI between groups. The MZ-MC group had significantly lower average birth weight and birth length than the MZ-DC and DZ groups (**Table 1**).

There were no significant differences in absolute or relative discordance of EFW or HC at second or third trimester ultrasounds between groups (**Tables 2-3, Figure 1**). Similarly, there were no significant differences in absolute or relative discordance of BPD, FL, and AC at second or third trimester ultrasound between groups (**Tables 2-3**).

At birth, there were no significant differences in absolute or relative discordance of birth weight, birth length, or birth head circumference between groups (**Table 4, Figure 1**). On MRI at term, the DZ group had significantly higher intracranial volume discordance overall (**Table 4, Figure 2**) as well as significantly greater discordance of head circumference at the MRI visit than the MZ-MC group (**Table 4, Figure 2**).

When placed in zygosity groups (without the additional factor of chorionicity), MZ twins had birth weight discordance comparable to that of DZ twins (MZ: $243.31\text{g} \pm 179.04$; DZ: $241.46\text{g} \pm 188.32$; $p = 0.9630$) while having lower intracranial volume discordance (MZ: $20473.91\text{mm}^3 \pm 12838.63$; DZ: $33364.16\text{mm}^3 \pm 24541.19$; $p = 0.0039$).

We sought to determine if relative discordance in head size was related to relative discordance in body size. There were no significant correlations between discordance of EFW and discordance of head size metrics (BPD, HC) in the second or third trimester (**Table 5**), although there were significant correlations between discordance of EFW and discordance of other measures of body size (AC, FL) in some or all three zygosity-chorionicity groups (data not shown). Postnatally, there was no significant correlation between birth weight discordance and birth head circumference discordance in any zygosity/chorionicity group (**Table 5**).

Longitudinal analysis of discordance of weight and head circumference at second trimester ultrasound, third trimester ultrasound, and birth was performed for the subset of twins with scans at all three visits ($N=41$ pairs). There were no significant differences in the changing pattern of weight discordance (heterogeneity of slopes = 0.50; $p = 0.7383$) or head circumference discordance (heterogeneity of slopes = 1.29; $p = 0.2826$) over time between the MZ-MC, MZ-DC, and DZ groups.

There were no significant differences in the rates of prenatal complications, including preterm labor, preterm premature rupture of membranes, pregnancy hypertension, perinatal diabetes, bleeding, placenta previa, infection during pregnancy, and advanced maternal age, between the MZ-MC, MZ-DC, and DZ groups (data not shown). There was also no significant difference in the distribution of pairs with birth weight discordance $<15\%$ and $\geq 15\%$ between the three groups. ($\% \geq 15\%$ MZ-MC: 68.18%; MZ-DC: 88.24%; DZ: 81.63%; $p = 0.3244$). The

rates of the same prenatal complications were then compared in groups of $<15\%$ and $\geq 15\%$ birth weight discordant pairs rather than zygosity-chorionicity groups. The rate of pregnancy hypertension differed between groups (BWD $<15\%$: 8 with pregnancy hypertension (11.43%); BWD $\geq 15\%$: 6 (33.33%); $p=0.0342$). There were no significant differences in rates of perinatal complications and proxies for prematurity, including total number of perinatal complications for pair, gestational age at birth, total days in NICU, total days of supplemental oxygen, and total days of intubation, between the three zygosity-chorionicity groups.

Discussion

The primary goal of our study was to examine the effects of zygosity and chorionicity on discordance of brain size in twins before and in the first weeks after birth. We found that there were no significant differences between MZ-MC, MZ-DC, and DZ twins in discordance of brain and body size measures in the second or third trimester or at birth. There were no significant correlations between discordance of brain and body size measures before or at birth in any zygosity-chorionicity group. Longitudinally, there were no differences in the changing pattern of weight or head circumference discordance over time between the MZ-MC, MZ-DC, and DZ groups. Rates of most prenatal and perinatal complications were similar for zygosity-chorionicity groups and birth weight discordance groups. Finally, we found head circumference and intracranial volume were significantly more discordant in DZ twins at the term MRI visit.

The similarity of discordance of HC and BPD across twin groups is consistent with previous studies (Persson and Grennert, 1979; Gilmore et al., 1996; Charlemaïne et al., 1997, Charlemaïne et al., 2000). MZ-MC twins had greater relative discordance of birth weight compared to the other groups; while not statistically significant in our study, this is consistent with previous studies (Corey et al., 1979; Dube et al., 2002; Gonzalez-Quintero et al., 2003; Race et al., 2006; Hack et al., 2008).

Head circumference and intracranial volume were significantly more discordant in DZ twins at the term MRI visit, which occurred on average 5.5 weeks after birth, while there were no significant differences in head circumference between groups observed at birth. This is consistent with a previous study that found correlations of head circumference at birth to be similar between MZ and DZ twins, while correlations increased in the MZ twins at one month of age and thereafter, but did not change for DZ twins (Chen et al., 1990). A subsequent study

found correlations of head circumference at birth to be greater in MZ twins compared to DZ twins (Livshits et al., 2000). Brain growth in the early postnatal period is very rapid, especially growth of cortical gray matter (Gilmore et al., 2007). There is also evidence that the genetic contribution to brain size and cerebral cortex thickness increases with age, at least in older children (Wallace et al., 2006; Lenroot et al., 2007). In the weeks between birth and MRI, outside of the uterine environment, genetic factors may begin to outweigh environmental factors in the developmental trajectory, with the brain size of DZ twins becoming more discordant, and that of MZ twins becoming more concordant.

In the prenatal period and at birth, MZ twins have discordance of brain and body size that is similar to that observed in DZ twins, in spite of being genetically identical. This may be due to placental factors that make MZ twins more discordant than one might expect given their identical genetic makeup. MC twins face additional challenges in utero. Twin-twin transfusion syndrome complicates 10-15% of MC twin pregnancies (Duncan et al., 1997). Placental factors, such as unequal placental sharing and “placental crowding,” have been associated with discordance and small for gestational age (SGA) size in MC twins (Fick et al., 2006; Blickstein et al., 2006; Lewi et al., 2007). Even DC twins may be affected by placental factors as peripheral cord insertion (suggestive of a spatially limited intrauterine compartment) has been associated with SGA infants and discordant growth in both MC and DC twins (Redline et al., 2001).

Discordant growth of the brain may also arise for other reasons. Though presumed to be genetically identical, MZ twins may have true genetic differences related to epigenetic dissimilarity, including DNA methylation (Singh et al., 2002; Fraga et al., 2005, Mill et al., 2006) or differences in DNA copy number (Bruder et al., 2008).

We found no significant correlation between head size discordance and estimated or measured weight discordance before or at birth in any zygosity-chorionicity group, although there was a significant positive correlation between birth weight discordance and birth head circumference discordance in the overall sample. A past study found no correlation between interpair differences of third trimester BPD and birth weight (Persson and Grennert, 1979), while another found a significant positive correlation between interpair difference of BPD and percent birth weight discordance (Brown et al., 1987). Sensitivities of 9-35%, 68%, and 35.7-57.1% have been reported for a range of values of BPD discordance as predictors of birth weight discordance (Erkkola et al., 1985; Brown et al., 1987; Shah et al., 1994), demonstrating the lack of a close correlation between BPD discordance and overall growth discordance as seen in our findings.

Our finding of a significantly higher incidence of pregnancy hypertension in twins with \geq 15% birth weight discordance concurs with a previous study that found an association between twin birth weight discordance and maternal hypertensive disorders, as well as smoking and increased maternal age (Sannoh et al., 2003). Monochorionicity has been associated with adverse outcomes such as preterm delivery, increased rates of NICU admission and longer NICU stays, neurological and respiratory morbidities, and perinatal mortality (Sebire et al., 1997; Victoria et al., 2001; Dube et al., 2002; Acosta-Rojas et al., 2007; Hack et al., 2008). Birth weight discordance has similarly been associated with a greater incidence of adverse perinatal outcomes (Hollier et al., 1999; Amaru et al., 2004; Vergani et al., 2004). We found no differences in rates of perinatal morbidities between zygosity-chorionicity groups or birth weight discordance groups, but our ability to detect differences was limited by our sample size.

In summary, we found that MZ twins had discordance of brain size in the late prenatal at birth similar to those observed in DZ twins while after several weeks of postnatal development MZ twin brain size was significantly less discordant than DZ twins. Prenatal discordances of brain size in MZ twins may be the result of variations in placental blood flow that would tend to violate the equal environment assumption of twin studies. However, discordances of brain size after even a few weeks of postnatal brain development appear to reflect “normalization” of developmental trajectories, with MZ twins becoming more concordant. We plan to follow this cohort into childhood to better understand the developmental trajectories of brain development in twins.

Conflict of Interest Statement

We have no conflicts of interest to disclose.

Acknowledgements

This research is supported by MH070890 (JHG) and the Doris Duke Charitable Foundation (NM).

References

1. Freitag CM. The genetics of autistic disorders and its clinical relevance: a review of the literature. *Mol Psychiatry* 2007; 12:2-22.
2. Kjeldsen MJ, Kyvik KO, Christensen K, Friis ML. Genetic and environmental factors in epilepsy: a population-based study of 11900 Danish twin pairs. *Epilepsy Res* 2001; 44:167-78.
3. Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence of from a meta-analysis of twin studies. *Arch Gen Psychiatry* 2003; 60:1187-92.
4. Martin N, Scourfield J, McGuffin P. Observer effects and heritability of childhood attention-deficit hyperactivity disorder. *Br J Psychiatry* 2002; 180:260-5.
5. Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ. A test of the equal-environment assumption in twin studies of psychiatric illness. *Behav Genet* 1993; 23:21-7.
6. Phillips DIW. Twin studies in medical research: can they tell us whether diseases are genetically determined? *Lancet* 1993; 341:1008-9.
7. Machin GA. Twins and their disorders. In: Reed GB, Claireaux AE, Cockburn F, editors. *Diseases of the fetus and newborn*. London: Chapman & Hall; 1995. p. 201-25.
8. Kingdom JCP, Nevo O, Murphy KE. Discordant growth in twins. *Prenat Diagn* 2005; 25:759-65.
9. Bagchi S, Salihu MH. Birth weight discordance in multiple gestations: Occurance and outcome. *J Obstet Gynaecol* 2006; 26:291-296
10. Adegbite AL, Castille S, Ward S, Bajoria R. Neuromorbidity in preterm twins in relation to chorionicity and discordant birth weight. *Am J Obstet Gynecol* 2004; 190:156-63.

11. Blickstein I, Keith LG. Neonatal mortality rates among growth-discordant twins, classified according to the birth weight of the smaller twin. *Am J Obstet Gynecol* 2004; 190:170-4.
12. Yinon Y, Mazkereth R, Rosentzweig N, Jarus-Hakak A, Schiff E, Simchen MJ. Growth restriction as a determinant of outcome in preterm discordant twins. *Obstet Gynecol* 2005; 105:80-4.
13. Hack KEA, Derks JB, Elias SG, Franx A, Roos EJ, Voerman SK, Bode CL, et al. Increased perinatal mortality and morbidity in monochorionic versus dichorionic twin pregnancies: clinical implications of a large Dutch cohort study. *Br J Obstet Gynaecol* 2008; 115:58-67.
14. Corey LA, Nance WE, Kang KW, Christian JC. Effects of type of placentation on birthweight and its variability in monozygotic and dizygotic twins. *Acta Genet Med Gemellol* 1979; 28:41-50.
15. Dubé J, Dodds L, Armson BA. Does chorionicity or zygosity predict adverse perinatal outcomes in twins? *Am J Obstet Gynecol* 2002; 186:579-83.
16. Gonzalez-Quintero VH, Luke B, O'Sullivan MJ, Misiunas R, Anderson E, Nugent C, et al. Antenatal factors associated with significant birth weight discordance in twin gestations. *Am J Obstet Gynecol* 2003; 189:813-7.
17. Race JP, Townsend GC, Hughes TE. Chorion type, birthweight discordance and tooth-size variability in Australian monozygotic twins. *Twin Res Hum Genet* 2006; 9:285-91.
18. Chauhan SP, Shields D, Parker D, Sanderson M, Scardo JA, Magann EF. Detecting fetal growth restriction or discordant growth in twin gestations stratified by placental chorionicity. *J Reprod Med* 2004; 49:279-84.

19. Blickstein I, Mincha S, Goldman RD, Machin GA, Keith LG. The Northwestern twin chorionicity study: testing the “placental crowding” hypothesis. *J Perinat Med* 2006; 34:158-61.
20. Persson PH, Grennert L. The intrauterine growth of the biparietal diameter of twins. *Acta Genet Med Gemellol* 1979; 28:271-277.
21. Gilmore JH, Perkins DO, Kleiwer MA, Hage ML, Silva SG, Chescheir NC, et al. Fetal brain development of twins assessed in utero by ultrasound: implications for schizophrenia. *Schizophr Res* 1996; 19:141-9.
22. Charlemaine C, Duyme M, Dubreuil E, Clauzel JP, Brossard Y, Aurengo A, et al. Biparietal diameter in twins at gestational weeks 18-32 – differences and similarities. *J Reprod Med* 1997; 42:725-30.
23. Charlemaine C, Duyme M, Ville Y, Aurengo A, Tremblay R, Frydman R, et al. Fetal biometric parameters, twin type and birth weight difference: a longitudinal study. *Eur J Obstet Gynaecol Reprod Biol* 2000; 93:27-32.
24. Hadlock FP, Harrist RB, Carpenter RJ, Deter RL, Park SK. Sonographic estimation of fetal weight: the value of femur length in addition to head and abdomen measurements. *Radiology* 1984; 150:535-40.
25. Prastawa M, Gilmore JH, Lin W, Gerig G. Automatic segmentation of MR images of the developing newborn brain. *Med Image Anal* 2005; 9:457-66.
26. Gilmore JH, Lin W, Prastawa MW, Looney CB, Vetsa YS, Knickmeyer RC, et al. Regional gray matter growth, sexual dimorphism, and cerebral asymmetry in the neonatal brain. *J Neurosci* 2007; 27:1255-60.

27. Chen CJ, Yu MW, Wang CH, Tong SL, Tein M, Lee TY, et al. Chronological changes in genetic variance and heritability of anthropometric characteristics among Chinese twin infants. *Acta Genet Med Gemellol* 1990; 6:726-36.
28. Livshits G, Peter I, Vainder M, Hauspie R. Genetic analysis of growth curve parameters of body weight, height and head circumference. *Ann Hum Biol* 2000; 27: 299-312.
29. 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. *J Child Psychol Psychiatr* (2006) 47: 987-993.
30. 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. *Huma Brain Mapp* 2007 (epub).
31. Duncan KR, Denbow ML, Fisk NM. The aetiology and management of twin-twin transfusion syndrome. *Prenat Diagn* 1997; 17:1227-36.
32. Fick AL, Feldstein VA, Norton ME, Wassel Fyr C, Caughey AB, Machin GA. Unequal placental sharing and birth weight discordance in monochorionic diamniotic twins. *Am J Obstet Gynecol* 2006; 195:178-83.
33. Lewi L, Cannie M, Blickstein I, Jani J, Huber A, Hecher K, Dymarkowski S, Gratacos E, Lewi P, Deprest J. Placental sharing, birtweight discordance, and vascular anastomoses in monochorionic diamniotic twin placentas. *Am J Obstet Gynecol* 2007; 197:587.e1-587.e8.
34. Redline RW, Shah D, Sakar H, Schluchter M, Salvator A. Placental lesions associated with abnormal growth in twins. *Pediatr Dev Pathol* 2001; 4:473-81.

35. Singh SM, Murphy B, O'Reilly R. Epigenetic contributors to the discordance of monozygotic twins. *Clin Genet* 2002; 62:97-103.
36. Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, Heine-Suner D, Cigudosa JC, Urioste M, Benitez J, Boix-Chornet M, Snachez-Aguilera A, Ling C, Carlsson E, Poulsen P, Vaag A, Stephan Z, Spector TD, Wu YZ, Plass C, Esteller M. Epigenetic differences arise during the lifetime of monozygotic twins. *PNAS* 2005; 102: 10604-10609.
37. Mill J, Dempster E, Caspi A, Williams B, Moffitt T, Craig I. Evidence for monozygotic twin (MZ) discordance in methylation level at two sites in the promoter regions of the catechol-O-methyltransferase (COMT) gene. *Am J Med Genetics Part B (Neuropsychiatric Genetics)* 2006; 141B:421-425.
38. Bruder CEG, Piotrowski A, Gijbbers AACJ, Andersson R, Erickson S, Diaz de Stahl T, Menzel U, Sandgren J, von Tell D, Poplawski A, Crowley M, Crasto C, Partridge EC, Tiwari H, Allison DB, Komorowski J, van Ommen GJB, Boomsma DI, Pedersen NL, den Dunnen JT, Wirdefeldt K, Dumanski JP. Phenotypically concordant and discordant monozygotic twins display different DNA copy-number-variation profiles. *Am J Hum Genet* 2008; 82:1-9.
39. Brown CEL, Guzick DS, Leveno KJ, Santos-Ramos R, Whalley PJ. Prediction of discordant twins using ultrasound measurement of biparietal diameter and abdominal perimeter. *Obstet Gynecol* 1987; 70:677-81.
40. Erkkola R, Ala-Mello S, Piironen O, Kero P, Sillanpaa M. Growth discordancy in twin pregnancies: a risk factor not detected by measurements of biparietal diameter. *Obstet Gynecol* 1985; 66:203-6.

41. Shah YG, Sherer DM, Gragg LA, Casaceli CJ, Woods JR. Diagnostic accuracy of different ultrasonographic growth parameters in predicting discordancy in twin gestation: a different approach. *Am J Perinatol* 1994; 11:199-204.
42. Sannoh S, Demissie K, Balasubramanian B, Rhoads GG. Risk factors for intraintra-pair birth weight discordance in twins. *J Matern Fetal Neonatal Med* 2003; 13:230-6.
43. Sebire NJ, Snijders RJM, Hughes K, Sepulveda W, Nicolaides KH. The hidden mortality of monochorionic twin pregnancies. *Br J Obstet Gynaecol* 1997; 104:1203-7.
44. Victoria A, Mora G, Arias F. Perinatal outcome, placental pathology, and severity of discordance in monochorionic and dichorionic twins. *Obstet Gynecol* 2001; 97:310-5.
45. Acosta-Rojas R, Becker J, Munoz-Abellana B, Ruiz C, Carreras E, Gratacos E. Twin chorionicity and the risk of adverse perinatal outcome. *Int J Gynecol Obstet* 2007; 96:98-102.
46. Hollier LM, McIntire DD, Leveno KJ. Outcome of twin pregnancies according to intraintra-pair birth weight differences. *Obstet Gynecol* 1999; 94:1006-10.
47. Amaru RC, Bush MC, Berkowitz RL, Lapinski RH, Gaddipati S. Is discordant growth in twins an independent risk factor for adverse neonatal outcome? *Obstet Gynecol* 2004; 103:71-6.
48. Vergani P, Locatelli A, Ratti M, Scian A, Zangheri G, Pezzullo JC, et al. Predictors of adverse perinatal outcome in twins delivered at < 37 weeks. *J Matern Fetal Neonatal Med* 2004; 16:343-7.

Table 1: Sample Demographics

		MZ-MC	MZ-DC	DZ(-DC)	Overall	p-value*
Number of pairs		22	17	49	88	
Maternal ethnicity						
N (%)	White	17 (77.27%)	11 (64.71%)	35 (71.43%)	63 (71.59%)	
	African-American or Black	4 (18.18%)	5 (29.41%)	14 (28.57%)	23 (26.14%)	
	Asian	1 (4.55%)	1 (5.88%)	0 (0%)	2 (2.27%)	0.3452
Gender						
N (%)	Male-male	9 (40.91%)	9 (52.94%)	27 (55.10%)	45 (51.14%)	
	Female-female	13 (59.09%)	8 (47.06%)	22 (44.90%)	43 (48.86%)	0.5456
Maternal age (years)						
Mean (SD)		31.41 (6.28)	27.71 (4.13)	30.45 (6.67)	30.16 (6.23)	0.1639
Gestational age at 2nd tr ultrasound (weeks)						
Mean (SD)		27.21 (6.09)	28.10 (7.91)	27.05 (6.64)	29.30 (6.70)	0.8581
Gestational age at 3rd tr ultrasound (weeks)						
Mean (SD)		31.70 (1.15)	32.18 (0.89)	31.81 (1.00)	31.84 (1.02)	0.4525
Gestational age at birth (weeks)						
Mean (SD)		34.68 (2.57)	35.67 (2.79)	35.83 (2.31)	35.51 (2.50)	0.1924
Gestational age at MRI (weeks)						
Mean (SD)		40.59 (1.79)	40.61 (1.73)	41.48 (2.27)	41.09 (2.09)	0.1491
Days from birth to MRI (days)						
Mean (SD)		41.41 (19.03)	34.59 (22.36)	39.71 (22.85)	39.15 (21.75)	0.6064
Average birth weight (g)						
Mean (SD)		2152.11 (540.32)	2423.35 (542.8)	2461.46 (516.24)	2375.79 (540.72)	0.0055**
Average birth length (cm)						
Mean (SD)		44.37 (3.68)	47.26 (2.88)	46.28 (3.33)	45.98 (3.48)	0.0006***

* Demographic factors were tested with three-group ANOVA except for maternal ethnicity and gender, which were tested with Fisher's exact test. Overall p-values shown.

** Pairwise comparisons: MZ-MC vs. MZ-DC: $p=0.0256$; MZ-MC vs. DZ-DC: $p=0.0015$; MZ-DC vs. DZ-DC: $p=0.7179$.

*** Pairwise comparisons: MZ-MC vs. MZ-DC: $p=0.0003$; MZ-MC vs. DZ-DC: $p=0.0022$; MZ-DC vs. DZ-DC: $p=0.1520$.

Table 2: Absolute and relative discordance of biometrics at second trimester

	Discordance type	MZ-MC	MZ-DC	DZ(-DC)	Overall	p-value (ANOVA)
Number of pairs		12	9	30*	51	
EFW	Absolute (g)	42.25 (24.77)	24.44 (18.89)	37.97 (36.36)	36.56 (31.42)	0.4172
Mean (SD)	Relative (%)	0.08 (0.05)	0.06 (0.04)	0.07 (0.06)	0.07 (0.05)	0.5455
HC	Absolute (cm)	0.44 (0.37)	0.59 (0.37)	0.55 (0.37)	0.53 (0.37)	0.6025
Mean (SD)	Relative (%)	0.02 (0.02)	0.03 (0.02)	0.03 (0.02)	0.03 (0.02)	0.6229
BPD	Absolute (cm)	0.16 (0.14)	0.21 (0.17)	0.16 (0.11)	0.17 (0.13)	0.5300
Mean (SD)	Relative (%)	0.03 (0.03)	0.04 (0.03)	0.03 (0.02)	0.03 (0.02)	0.4895
FL	Absolute (cm)	0.11 (0.06)	0.14 (0.11)	0.16 (0.15)	0.15 (0.13)	0.4516
Mean (SD)	Relative (%)	0.03 (0.02)	0.04 (0.03)	0.04 (0.04)	0.04 (0.03)	0.5167
AC	Absolute (cm)	0.90 (0.67)	0.38 (0.27)	0.68 (0.64)	0.68 (0.61)	0.1547
Mean (SD)	Relative (%)	0.05 (0.04)	0.02 (0.02)	0.04 (0.03)	0.04 (0.03)	0.1512

* 29 DZ pairs had EFW and AC measurements.

Table 3: Absolute and relative discordance of biometrics at third trimester

Discordance type		MZ-MC	MZ-DC	DZ(-DC)	Overall	p-value (ANOVA)
Number of pairs		18	11	40	69	
EFW Mean (SD)	Absolute (g)	171.00 (144.66)	167.73 (141.16)	143.08 (120.21)	154.29 (128.95)	0.7023
	Relative (%)	0.09 (0.08)	0.08 (0.07)	0.07 (0.06)	0.08 (0.07)	0.5272
HC Mean (SD)	Absolute (cm)	1.17 (0.73)	0.49 (0.50)	1.00 (1.10)	0.96 (0.95)	0.1681
	Relative (%)	0.04 (0.02)	0.02 (0.02)	0.03 (0.03)	0.03 (0.03)	0.1099
BPD Mean (SD)	Absolute (cm)	0.31 (0.31)	0.23 (0.10)	0.38 (0.27)	0.34 (0.27)	0.2459
	Relative (%)	0.04 (0.04)	0.03 (0.01)	0.05 (0.03)	0.04 (0.03)	0.2546
FL Mean (SD)	Absolute (cm)	0.27 (0.18)	0.17 (0.15)	0.20 (0.15)	0.22 (0.16)	0.2455
	Relative (%)	0.04 (0.03)	0.03 (0.02)	0.03 (0.02)	0.04 (0.03)	0.1980
AC Mean (SD)	Absolute (cm)	1.24 (0.97)	1.45 (0.94)	1.42 (1.48)	1.38 (1.27)	0.8672
	Relative (%)	0.04 (0.03)	0.05 (0.03)	0.05 (0.04)	0.05 (0.04)	0.9227

Table 4: Absolute and relative discordance of biometrics at birth and at MRI visit

Discordance type		MZ-MC	MZ-DC	DZ(-DC)	Overall	p-value (ANOVA)
Number of pairs		22*	17**	49***	88	
Birth weight	Absolute (g)	271.14 (193.57)	207.29 (156.57)	241.46 (188.32)	242.29 (183.16)	0.5633
	Mean (SD)	Relative	0.12 (0.08)	0.08 (0.06)	0.09 (0.07)	0.10 (0.07)
Birth length	Absolute (cm)	1.85 (1.60)	2.09 (1.97)	1.51 (1.30)	1.70 (1.51)	0.3835
	Mean (SD)	Relative	0.04 (0.03)	0.04 (0.04)	0.03 (0.03)	0.04 (0.03)
Birth HC	Absolute (cm)	1.19 (1.10)	0.86 (0.66)	0.96 (0.89)	1.00 (0.91)	0.5051
	Mean (SD)	Relative	0.04 (0.03)	0.03 (0.02)	0.03 (0.03)	0.03 (0.03)
ICV	Absolute (mm ³)	21716.66 (13131.61)	18865.64 (12659.92)	33364.16 (24541.19)	27651.44 (21112.94)	0.0143 ¹
	Mean (SD)	Relative	0.05 (0.03)	0.04 (0.03)	0.07 (0.05)	0.06 (0.04)
HC at MRI	Absolute (cm)	0.45 (0.43)	0.57 (0.58)	0.87 (0.75)	0.71 (0.67)	0.0310 ³
	Mean (SD)	Relative	0.01 (0.01)	0.02 (0.02)	0.02 (0.02)	0.02 (0.02)

* 21 MZ-MC pairs with birth length and birth head circumference

** 15 MZ-DC pairs with birth length; 16 MZ-DC pairs with birth head circumference

*** 48 DZ pairs with birth weight; 47 with birth length and birth head circumference

¹ Pairwise: MZ-MC vs. MZ-DC: 0.6650; MZ-MC vs. DZ: **0.0281**; MZ-DC vs. DZ: **0.0131**

² Pairwise: MZ-MC vs. MZ-DC: 0.6306; MZ-MC vs. DZ: 0.0578; MZ-DC vs. DZ: **0.0235**

³ Pairwise: MZ-MC vs. MZ-DC: 0.5658; MZ-MC vs. DZ: **0.0138**; MZ-DC vs. DZ: 0.1068

⁴ Pairwise: MZ-MC vs. MZ-DC: 0.5638; MZ-MC vs. DZ: **0.0183**; MZ-DC vs. DZ: 0.1298

Table 5: Correlation of relative discordance of weight and head size at second trimester, third trimester, birth, and term MRI.

		MZ-MC	MZ-DC	DZ(-DC)	Overall
2nd trimester	EFW discordance vs. HC discordance	0.1486	0.6074	0.1986	0.2100
	EFW discordance vs. BPD discordance	0.2291	0.8251	0.9480	0.4755
3rd trimester	EFW discordance vs. HC discordance	0.3587	0.3901	0.1661	0.2286
	EFW discordance vs. BPD discordance	-0.1640	0.3906	0.0207	-0.0375
Birth/Term MRI	BW discordance vs. birth HC discordance	0.4072	0.4546	0.1923	0.3163¹

Pearson correlation coefficients are shown; statistically significant coefficients are in bold type.

¹p=0.0034; N=84

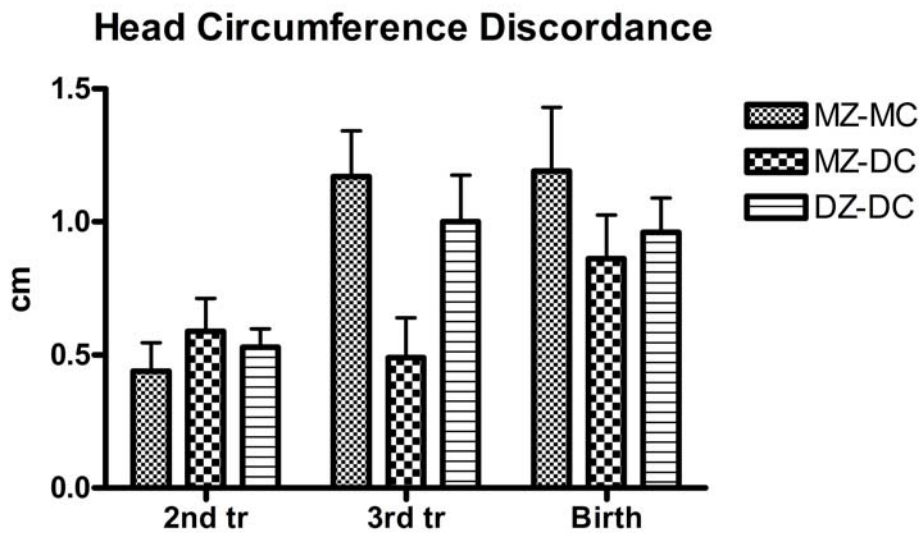


Figure 1a: Discordance of head circumference. There were no significant differences in absolute intrapair discordance of head circumference at 2nd trimester, 3rd trimester, or birth between groups. Data shown in Tables 2-4.

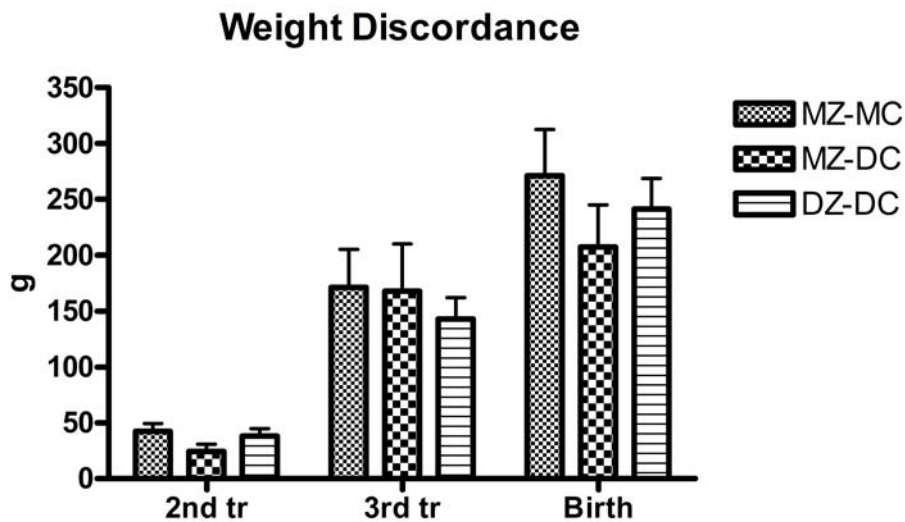


Figure 1b: Discordance of estimated fetal weight and birth weight. There were no significant differences in absolute intrapair discordance of weight at 2nd trimester, 3rd trimester, or birth between groups. Data shown in Tables 2-4.

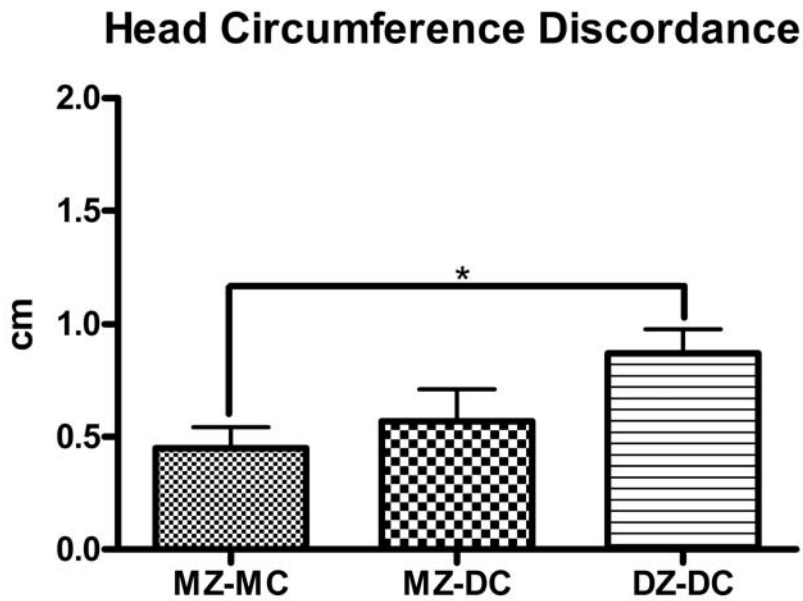


Figure 2a: Discordance of head circumference at MRI. ANOVA: $p = 0.0310$; *DZ-DC vs MZ-MC: $p = 0.0138$.

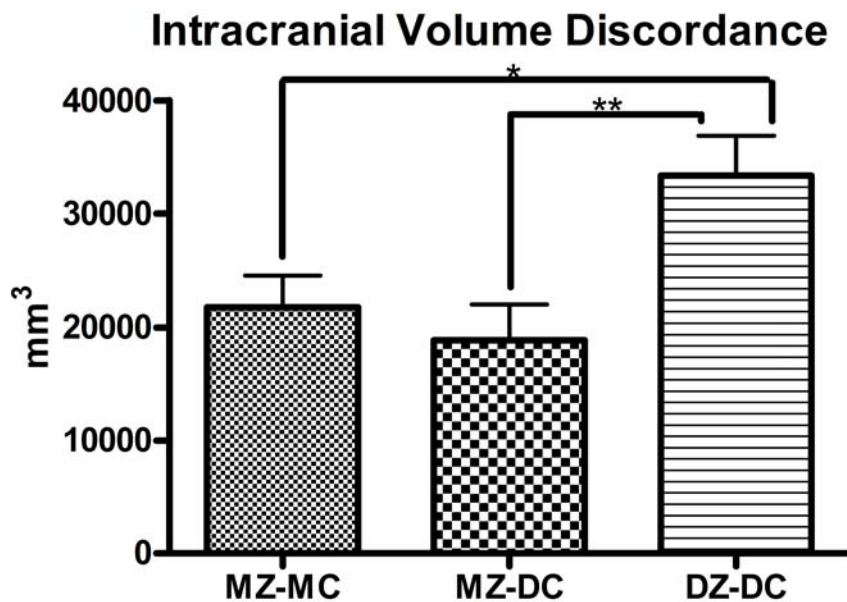


Figure 2b: Discordance of intracranial volume at MRI visit. Overall ANOVA: $p = 0.0143$; *DZ-DC vs. MZ-MC: $p = 0.0281$; **DZ-DC vs. MZ-DC: $p = 0.0131$.