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Twin–singleton differences in brain structure using structural equation modelling

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Summary

Twin studies are important to investigate genetic influences on variation in human brain morphology in health and disease. However, the twin method has been criticized for its alleged non-generalizability due to differences in the intrauterine and family environment of twins, compared with singletons. To test whether twin–singleton differences complicate interpretation of genetic contributions on variation in brain volume, brains from 112 pairs of twins and 34 of their siblings with a mean (standard deviation) age of 30.7 (9.6) years were scanned using MRI. The influence of birth order, zygosity and twin–sibling differences on brain volume measures was analysed using maximum-likelihood model fitting. Variances were homogeneous across birth order, zygosity and twin–singleton status. Irrespective of zygosity, intracranial volume was smaller in second-born twins compared with first-born twins and compared with siblings. Grey matter volume was smaller in second-born twins compared with first-born twins. White matter was smaller in twins compared with siblings. Differences in grey and white matter between these groups were no longer significant after correction for intracranial volume. Total brain, and lateral and third ventricle volumes were comparable in twins and singletons. In conclusion, second-born twins have a smaller intracranial volume than their first-born co-twins and siblings. This suggests aberrant early brain development in second-born twins, which is consistent with the suboptimal pre- and perinatal environment related to birth order in twins. Since other brain volume measures were comparable between the groups, twin studies can provide reliable estimates of heritabilities in brain volume measures and these can be generalized to the singleton population.

Keywords: twins; structural equation modelling; MRI; brain; birth order

Abbreviations: DOS = dizygotic opposite-sex twins; DZ = dizygotic; DZF = dizygotic female twins; DZM = dizygotic male twins; MZ = monozygotic; MZF = monozygotic female twins; MZM = monozygotic male twins; SF = female sibling; SM = male sibling

Introduction

Twins studies are considered important to investigate genetic influences on variation in human brain morphology. Several studies have investigated quantitatively the contribution of genetic and environmental influences to individual differences in human brain structure (Bartley *et al*., 1997; Carmelli *et al*., 1998; Lohmann *et al*., 1999; Le Goualher *et al*., 2000; Pennington *et al*., 2000; Pfefferbaum *et al*., 2000; Baaré *et al*., 2001*a*). Moreover, twin studies have shown genetic and environmental influences on the interaction between brain structure and psychiatric disease (Reveley *et al*., 1982; Suddath *et al*., 1990; Baaré *et al*., 2001*b*).

The twin method has sometimes been criticized for its alleged non-generalizability due to differences in intrauterine and family environment of twins, compared with singletons. As foetuses, twins share the womb and prenatal nutrition, and compete for the best position during labour. The intrauterine environment may therefore be considered as suboptimal compared with that of singletons, with the greatest disadvantage for the second born of a (monozygotic) twin pair (Price *et al*., 1950). In addition, it has been argued that family environments in which twins are reared can be suboptimal compared with those of singletons. Some studies have shown

that physical likeness, limitation of resources and competition may lead to negative influences on the cognitive development of at least one twin member (Hay *et al*., 1983). However, in a large sample of 3-year-old twins, it was found that they had similar, or even lower, levels of behavioural and emotional problems than singletons (Van den Oord *et al*., 1996).

Evidence for differences between twins and singletons has been suggested in a few studies for cognitive measures (Hay *et al*., 1983; Nathan *et al*., 1984), raising concerns regarding generalizations towards the singleton population (Vandenberg, 1984). However, these studies generally compared twins with genetically unrelated singletons, which complicates the generalizability of the findings. Recently, using an extended twin design (Posthuma and Boomsma, 2000; Posthuma *et al*., 2000*a*), a study was completed in which monozygotic and dizygotic twins were compared with their own siblings on intellectual ability, providing perfectly matched genetic and familial environments (Posthuma *et al*., 2000*b*). No evidence was found for differences in intellectual ability between twins and their siblings. This suggests that twin studies can provide reliable estimates of heritabilities, which can be generalized to the singleton population, at least with respect to intellectual abilities. Similarly, comparison of twins and their non-twin relatives showed no differences in psychiatric symptoms (Kendler *et al*., 1995). However, it is not known whether twin studies provide reliable estimates of heritabilities of brain structure. Studies examining the relative contributions of genetic and non-genetic factors to structural brain volume in health and disease rely on the assumption that brains of twins are comparable with those of singletons. To test whether twin–singleton differences complicate interpretation of genetic contributions to variation in brain structure, brain volume measures from pairs of twins and their siblings were compared.

Methods

Subjects

A total number of 258 family members from 112 families participated in the study after written consent was obtained (Baaré *et al*., 2001*a*). They consisted of 33 monozygotic (MZ) male (MZM), 17 dizygotic (DZ) male (DZM), 21 MZ female (MZF), 20 DZ female (DZF), 21 DZ opposite-sex (DOS) twin pairs and 19 male (SM) and 15 female (SF) full siblings. Twins were recruited from the (healthy) twin sample of the Department of Psychiatry of the University Medical Centre Utrecht, The Netherlands, and The Netherlands Twin Registry (Boomsma, 1998). DNA fingerprinting using either the polymorphic markers D06S474, D07S1804, D07S1870, D12S811, D13S119, D13S126, D13S788, D20S119, D22S683, DXS1001 and ELN, or D13S317, VWA, D74520, D35158, TH01, TP0X, CSF1P0 and D55818 determined zygosity. Except for one twin pair, all twins and their siblings were reared together. Two twin pairs were born by Caesarean section delivery. Subjects with severe medical diseases were excluded. Mental and physical health was assessed by means of the Family Interview for Genetic Studies (Nurnberger *et al*., 1994) and a medical history inventory, respectively. Birth weight was traced in 219 subjects (in 96 first-born twins, in 95 second-born twins and in 28 siblings). It was based on the report of the mother and, when this was not available, on the subject's report. Subjects' consent was obtained according to the declaration of Helsinki. The Scientific and Ethical Committee of the University Medical Centre Utrecht, in which the study was performed, approved the study.

Brain imaging

MRIs were obtained on a 1.5 tesla Philips Gyroscan scanner at the University Medical Centre Utrecht. For volumetric analysis, a three-dimensional T_1-weighted, coronal FFE (spoiled gradient echo scan) of the whole head [TE (echo time) = 4.6 ms, TR (repetition time) = 30 ms, flip angle = 30°, 170–180 contiguous slices; $1 \times 1 \times 1.2$ mm^3 voxels], and a coronal DTSE (dual contrast turbo spin echo) of the whole brain (TE1 = 14 ms, TE2 = 80 ms, TR = 6350 ms, 120 contiguous slices; $1 \times 1 \times 1.6$ mm^3 voxels) were acquired.

Images were coded to ensure blindness for subject identification, zygosity and family membership. Image volumes were transformed into Talairach space (no scaling) and corrected for magnetic field inhomogeneities (Maes *et al*., 1997; Sled *et al*., 1998). Volumetric measurements were obtained using automated segmentation procedures and included intracranial, whole brain, grey and white matter of the cerebrum (excluding cerebellum and brainstem), and lateral and third ventricle volumes. Automatic segmentation software included histogram analysis algorithms, anatomical knowledge-based decision rules and series of mathematical morphological operators to connect all voxels of interest (Schnack *et al*., 2001*a*, *b*). Intracranial volume was segmented on DTSE scans. Whole brain volume was segmented on the three-dimensional FFE scans using a binary image of the intracranial volume as a mask. A plane through the fourth ventricle and the aqueduct limited the cerebellum. In lateral ventricle segmentation, automatic decision rules bridged connections not detectable and prevented 'leaking' into cisterns. The third ventricle was limited by coronal slices that clearly showed the anterior and posterior commissures; the upper boundary was a plane through the plexus choroideus ventriculi tertii in the midsagittal slice perpendicular to this slice. Segmented intracranial, whole brain and lateral and third ventricle volumes were checked visually and edited if necessary. The segmentation procedures yielded highly reliable volume measurements, with inter-rater intraclass correlations all above 0.96.

Statistical analysis

Structural equation modelling with Mx software (Neale, 1997) was used to estimate the contribution of birth order of the twins to mean scores of and variance in brain volumes, to

Table 1 *Age and brain volumes in monozygotic (MZ), dizygotic (DZ) twins and their siblings*

	MZM 1st*	MZM 2nd	DZM 1st	DZM 2nd	DOS 1st male	DOS 2nd male	SM	MZF 1st	MZF 2nd	DZF 1st	DZF 2nd	DOS 1st female	DOS 2nd female	SF
	(*n* = 33)	(*n* = 33)	(*n* = 17)	(*n* = 17)	(*n* = 9)	(*n* = 12)	(*n* = 19)	(*n* = 21)	(*n* = 21)	(*n* = 20)	(*n* = 20)	(*n* = 12)	(*n* = 9)	(*n* = 15)
Mean age in years	30.76	30.75	29.82	29.76	31.78	28.50	28.89	33.67	33.67	30.20	30.15	28.50	31.78	29.53
(standard deviation)	(9.71)	(9.69)	(7.04)	(7.06)	(14.75)	(11.39)	(4.79)	(11.80)	(11.80)	(8.55)	(8.55)	(11.38)	(14.75)	(4.90)
Brain volumes, mean (standard deviation) in ml														
Intracranium	1531.39	1515.93	1496.98	1426.42	1486.62	1502.56	1528.68	1354.37	1330.48	1339.63	1327.74	1379.65	1245.77	1376.90
	(109.89)	(118.61)	(72.00)	(81.85)	(95.51)	(99.93)	(111.15)	(108.64)	(127.83)	(104.74)	(118.77)	(88.14)	(83.28)	(115.73)
Total brain	1339.64	1330.73	1329.87	1265.28	1298.87	1313.96	1348.06	1183.31	1170.66	11.82.15	1178.94	1211.86	1118.23	1211.54
	(106.18)	(111.68)	(66.86)	(77.55)	(97.96)	(97.62)	(92.76)	(110.19)	(124.11)	(115.44)	(109.12)	(77.69)	(68.22)	(108.10)
Grey matter	676.17	670.41	663.77	629.22	667.07	681.10	681.55	612.90	599.19	614.31	612.72	639.68	580.96	624.42
	(64.59)	(69.36)	(43.85)	(41.15)	(44.39)	(73.16)	(51.66)	(68.76)	(77.48)	(59.17)	(53.44)	(55.49)	(54.78)	(58.83)
White matter	498.81	494.85	506.98	478.68	474.33	476.36	505.31	426.58	428.08	422.37	415.45	423.37	397.62	436.14
	(53.42)	(55.15)	(34.36)	(47.15)	(53.91)	(44.35)	(49.04)	(49.40)	(58.64)	(55.94)	(57.34)	(42.48)	(27.14)	(53.40)
Lateral ventricles	15.26	15.19	12.76	11.78	17.67	16.90	13.88	14.39	13.47	11.99	9.83	16.10	9.72	14.23
	(9.11)	(7.33)	(6.28)	(5.89)	(10.29)	(9.18)	(7.96)	(7.09)	(6.37)	(7.64)	(3.90)	(6.81)	(2.56)	(7.03)
Third ventricle	0.79	0.75	0.70	0.64	0.95	0.67	0.69	0.75	0.68	0.59	0.56	0.68	0.50	0.78
	(0.42)	(0.35)	(0.28)	(0.31)	(0.56)	(0.41)	(0.34)	(0.29)	(0.28)	(0.30)	(0.19)	(0.26)	(0.18)	(0.24)

*1st = first born; 2nd = second born.

test the assumption that the mean volumes (and variance) in MZ twins and DZ twins do not differ, and to test whether the mean volumes (and variance) of twins and siblings are significantly different. Models were fitted to the raw data using maximum-likelihood to estimate parameters. Hierarchic χ^2 tests were used to compare the fit of different models. Twice the difference between the log-likelihood of two models is distributed asymptotically as χ^2. The degrees of freedom for these tests are equal to the difference in parameters being estimated. Utilizing the principle of parsimony, the most restrictive model is accepted as the best fitting one in case the difference between a nested and a more comprehensive model is not significant (Neale and Cardon, 1992).

Four univariate nested models were fitted using this procedure (Posthuma *et al*., 2000*a*). In the first model (the control model), the variances for brain volumes of all twin members and all siblings were constrained to be equal. In addition, all covariances of twin–sibpairs, the covariance of two sibs within one family and the covariance of the DZ twins were set to be equal. The second model (to test birth order effects) is the same as the first model, with two extra equality constraints; one on the means of both members of the MZ twin pairs and another on the means of both members of the DZ twin pairs. The third model (to test zygosity effects) is the same as the second model, but further constrains the means of the MZ twin pairs and the DZ twin pairs to be equal. The fourth model (to test twin–sibling differences) is the same as the third model, but with an extra equality constraint on the means of all twins (MZ and DZ) and siblings.

Univariate models were fitted on all variables, with the effects of age and sex corrected for by means of a linear regression on the observed values of each of the dependent variables.

Post hoc analyses were done with intracranial volume as a covariant, when effects for total brain, grey and white matter or ventricular volumes were found to evaluate the specificity of the finding. Moreover, in case of a significant finding for birth order, the influence of birth weight was tested, by adding birth weight as covariate to the analyses.

Results

Means and standard deviations of brain volume measures are shown in Table 1. The volume estimates in the control model are shown in Table 2. The tests for equality of variances showed no evidence of differences in variance according to birth order, zygosity or twin–singleton status. In addition, no differences were found in DZ covariance and sibpair covariances.

The results for the univariate analyses of birth order, zygosity and twin–sibling differences on the means of the brain volume measures using the Mx software are shown in Table 3.

Table 2 *Brain volume estimates (in ml) under the constraints that variances are equal for all family members (control model)*

	Grand mean MZ 1st*	Grand mean MZ 2nd	Grand mean DZ 1st	Grand mean DZ 2nd	Grand mean sibs	Age effect per year	Male deviation
Intracranium	1380.52	1361.80	1359.04	1328.90	1387.83	–0.57	158.59
Total brain	1263.92	1253.56	1248.11	1228.75	1272.47	–2.18	137.41
Grey matter	711.68	702.87	704.31	690.65	711.05	–2.86	52.02
White matter	397.91	396.07	393.03	384.55	407.25	0.93	69.31
Lateral ventricles	11.65	10.25	11.13	9.14	10.23	0.09	1.22
Third ventricle	0.39	0.34	0.36	0.30	0.40	0.01	0.09

*1st = first born; 2nd = second born.

Table 3 *Influence of birth order, zygosity and twin–sibling differences on brain volumes*

	Model 2* birth order		Model 3 zygosity		Model 4a twin–sibling		Model 4b twin–1st sibling		Model 4c twin–2nd sibling	
	χ^2	Δd.f.	χ^2	Δd.f.	χ^2	Δd.f.	χ^2	Δd.f.	χ^2	Δd.f.
Intracranium	**11.986**	2	3.102	2	–	–	2.749	1	**9.321**	1
Total brain	5.85	2	1.363	1	3.488	1				
Grey matter	**7.233**	2	1.494	2	–	–	0.405	1	3.364	1
White matter	1.678	2	0.896	1	**4.263**	1				
Lateral ventricles	4.521	2	0.056	1	0.001	1				
Third ventricle	4.996	2	0.481	1	1.475					

*An increase in χ^2 of >3.841 for Δd.f. = 1 is significant at the 0.05 level; an increase in χ^2 of >5.991 for Δd.f. = 2 is significant at the 0.05 level; χ^2 values in bold indicate a significant influence of the factor (i.e. the model cannot be accepted); note that when the increase in χ^2 is not significant, the most restrictive model is accepted.

For intracranial volume, the more restrictive models revealed a significant difference in means due to birth order ($\chi^2 = 11.99$, Δd.f. = 2, $P < 0.05$). The second-born twins had smaller intracranial volumes than the first-born twins. Because birth order mattered, separate comparisons for first-born and second-born twins with siblings were made. The mean intracranial volume of the first-born twins did not differ from that of the siblings. However, the mean intracranial volume of the second-born twins was smaller than that of the siblings ($\chi^2 = 9.32$, Δd.f. = 1, $P < 0.05$). For grey matter volume, the more restrictive models revealed a significant difference in mean due to birth order ($\chi^2 = 7.23$, Δd.f. = 2, $P < 0.05$). The comparisons for first-born and second-born twins with siblings revealed no significant differences in mean grey matter volume, although grey matter volume of the second-born twins was smaller compared with that of the siblings. For white matter volume, the more restrictive models revealed a significant difference ($\chi^2 = 4.26$, Δd.f. = 1, $P < 0.05$). Mean white matter volume in twins, irrespective of birth order and zygosity, was smaller than in siblings.

Post hoc analyses revealed that the effects of grey and white matter in (second-born) twins compared with siblings were no longer significant after correction for intracranial volume.

For the total brain, and the lateral and third ventricular volumes, the more restrictive models caused no significant differences in χ^2. This means that the more restrictive models may all be accepted. Thus, the estimated means of first- and second-born twins, the estimated means of MZ twins and DZ twins, and those of twins compared with siblings were not significantly different with respect to total brain, and lateral and third ventricular volumes.

There was a significant correlation between birth weight and intracranial volume ($r = 0.23$, $P < 0.01$). Birth weight was lower in second-born twins (mean ± standard deviation birth weight 2455.3 ± 569.9 g) compared with first-born twins (2575.6 ± 567.8 g) ($\chi^2 = 6.404$, Δd.f. = 2, $P < 0.05$). Twins had a lower birth weight than siblings (3369.1 ± 591.2 g) ($\chi^2 = 59.124$, (d.f. = 1, $P < 0.0001$). When birth weight was added as covariant in the model, it did not influence the results, i.e. intracranial volume remained significantly smaller in the second-born compared with the first-born twins ($\chi^2 = 11.481$, Δd.f. = 2, $P < 0.05$).

Discussion

This study compared brain morphology between MZ and DZ twins with their non-twin siblings. No differences in (co)variances were found according to birth order, zygosity or twin–singleton status. However, a mean difference was found for intracranial volume, such that the second-born twins had a significantly smaller intracranial volume compared with the first-born twins and compared with their

siblings, whereas the intracranial volume of first-born twins did not differ from that of the siblings. Moreover, grey matter volume was smaller in the second-born twins compared with the siblings, and white matter was smaller in the twins, irrespective of birth order and zygosity, compared with the siblings. After correction for intracranial volume, the effects for grey and white matter were no longer significant. Mean values of total brain, and lateral and third ventricular volumes were not influenced by birth order or zygosity, and no evidence was found for twin–sibling differences.

The finding that, irrespective of zygosity, second-born twins had a smaller intracranial volume than first-born twins and their siblings suggests that brain growth is influenced by non-genetic factors during early brain development. Brain growth is thought to be the main factor influencing growth of the neurocranium in the first years of life (O'Rahilly and Müller, 1992; Sgouros *et al*., 1999). No effect of birth order on head circumference was reported earlier, but that finding was based on the inclusion of 10 pairs of MZ twins only (Tramo *et al*., 1998). Because no effects of zygosity were found, it is likely that environmental and not genetic factors resulted in the development of a relatively smaller head in second-born twins. Nutritional deficiency during the first trimester of gestation (Hulshoff Pol *et al*., 2000), and birth complications (McNeil *et al*., 2000; for a review, see Frangou and Murray, 1996), in schizophrenia as well as very preterm birth (Allin *et al*., 2001) have all been related to decreased brain volume. Twin gestations have a significantly higher rate of complications compared with singleton gestations, particularly with regard to preterm labour, pregnancy-induced hypertension and foetal death (Kovacs *et al*., 1989; Doyle, 1996), and the second-born twin seems to be particularly at risk. The overall mortality risk of second-born twins has been reported to be 8% greater than that of first-born twins. Mortality risks as a result of respiratory distress syndrome, intrauterine hypoxia and birth asphyxia, and congenital anomalies were 19–27% higher among second-born twins than among first-born twins (Fowler *et al*., 1991). Moreover, second-born twins were found to have a lower birth weight compared with first-born twins in a sample of 193 twins where 55% of the second born twin were male (Daniel *et al*., 2000) and in a sample of 2930 Dutch twins (Baal and Boomsma, 1998). Although, in our study, no direct comparison between obstetric complications and brain volume measures was made, it is likely that pre- and perinatal factors specific for second-born twins influenced intracranial volume in these subjects. Correcting for birth weight in the analysis did not change the finding that second-born twins had a smaller intracranial volume than the first-born twins. Because the effects were only found in the second-born twins and not in the first-born twins, suboptimal family environments that have been associated with twin rearing (Hay and O'Brien, 1983) are less likely to have influenced intracranial volume.

Twin–sibling differences were found for mean white matter volumes, with those of twins being smaller than their siblings, and grey matter volume, which was smaller in the second-born twins compared with siblings. However, after correction for intracranial volume, these effects on the means were no longer significant. This suggests that the difference in (second-born) twins compared with siblings is due predominantly to differences in intracranial volume and occurs early in brain development. Moreover, it implies that overall volumes of grey and white matter do not develop differently in twins and singletons.

No differences in mean values of total brain, and lateral and third ventricular volumes were found secondary to twin–sibling differences. Although total brain volume was somewhat smaller in twins compared with siblings, this finding did not reach significance. The comparability of both the variances and the means of brain volumes across twins and their siblings suggests that suboptimal pre-, peri- and possibly postnatal circumstances in twins and siblings do not differentially influence total brain, and lateral and third ventricle volumes in twins and singletons. Moreover, it suggests that twin studies can provide reliable estimates of heritability of these brain volumes and that these estimates can be generalized to the singleton population.

Whether the smaller intracranial volume in second-born twins has consequences for their subsequent cognitive and behavioural development remains to be determined. However, it is unlikely that the smaller intracranial volume in second-born twins implies such consequences. In a study that included a majority of the twins from this study, no differences in intelligence measured by the Wechsler Adult Intelligence Scale and birth order in twins were found (Posthuma *et al*., 2000*b*). Earlier findings suggested that twins recover from deficits in intellectual performance by 6–8 years of age (Wilson, 1979). Indeed, by the age of 11 years, no evidence for a relationship between the order of delivery of twins on their intelligence quotient as measured by a verbal reasoning task was found (Record *et al*., 1970). Finally, levels of behavioural and emotional problems were found to be similar, or even lower, in 3-year-old twins compared with singletons (Van den Oord *et al*., 1996).

Our findings suggest that twin studies can provide reliable estimates of heritabilities of brain volumes that can be generalized to the singleton population. Whether twin–sibling differences occur in particular brain areas such as in limbic, diencephalic and basal ganglia structures remains to be established in future studies.

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