Psychological Medicine, 2005, **35**, 1399–1409. © 2005 Cambridge University Press
doi:10.1017/S0033291705005167 Printed in the United Kingdom

Brain MRI abnormalities in schizophrenia: same genes or same environment?

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ABSTRACT

Background. Structural brain volume abnormalities are among the most extensively studied endophenotypes in schizophrenia. Bivariate genetic model fitting (adjusted to account for selection) was used to quantify the genetic relationship between schizophrenia and brain volumes and to estimate the heritability of these volumes.

Method. We demonstrated by simulation that the adjusted genetic model produced unbiased estimates for endophenotype heritability and the genetic and environmental correlations. The model was applied to brain volumes (whole brain, hippocampus, third and lateral ventricles) in a sample of 14 monozygotic (MZ) twin pairs concordant for schizophrenia, 10 MZ discordant pairs, 17 MZ control pairs, 22 discordant sibling pairs, three concordant sibling pairs, and 114 healthy control subjects.

Results. Whole brain showed a substantial heritability (88%) and lateral ventricles substantial common environmental effects (67%). Whole brain showed a significant genetic correlation with schizophrenia, whereas lateral ventricles showed a significant individual specific correlation with schizophrenia. There were significant familial effects for hippocampus and third ventricle, but the analyses could not resolve whether these were genetic or environmental in origin (around 30% each).

Conclusions. Using genetic model fitting on twin and sibling data we have demonstrated differential sources of covariation between schizophrenia and brain volumes, genetic in the case of whole brain volume and individual specific environment in the case of lateral ventricles.

INTRODUCTION

While twin and adoption studies have shown substantial genetic influences in the risk to develop schizophrenia (McGuffin *et al.* 1984; Kendler & Diehl, 1993; Cannon *et al.* 1998; Cardno *et al.* 2002), the identification of predisposing genes has been hampered by difficulties in detecting non-penetrant carriers and by uncertainties concerning the extent of locus heterogeneity (McDonald & Murphy, 2003). By studying the inheritance of endophenotypes we can increase the power to detect the genes involved by clarifying the pathways leading from genetic predisposition to clinical disorder (Gottesman & Gould, 2003). Endophenotypes are quantitative traits (pathophysiological markers) that share a substantial genetic component with the clinical disorder.

The identification of endophenotypes has been a major focus of schizophrenia research in

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https://doi.org/10.1017/S0033291705005167 Published online by Cambridge University Press

the last decade. The most extensively studied candidates include: event-related potential (ERP) components such as the P300, P50 and mismatch negativity (Freedman *et al.* 1996; Weisbrod *et al.* 1999; Bramon *et al.* 2004); neuropsychological functioning (Cannon *et al.* 2000); and structural brain abnormalities (McNeil *et al.* 2000; Baaré *et al.* 2001*a*; McDonald *et al.* 2002; Narr *et al.* 2002; Hulshoff Pol *et al.* 2004). These structural changes are well-established in schizophrenia. The most robust findings from meta-analyses include: increased total ventricular volume (Lawrie & Abukmeil, 1998; Wright *et al.* 2000); reduction in both whole brain and intracranial volume (Ward *et al.* 1996; Lawrie & Abukmeil, 1998); and reduced hippocampal and amygdalar volumes (Lawrie & Abukmeil, 1998; Nelson *et al.* 1998; Wright *et al.* 2000).

What these findings tell us about the aetiology of the illness and their usefulness as endophenotype markers remains elusive. One hypothesis is that there is an overlap in the neuro-developmental genes responsible for both the volume change and the development of the illness, i.e. the existence of a genetic correlation. Schizophrenia may involve genetically determined pathological processes of early brain development which continues to unfold as the brain matures through neuronal loss and synaptic pruning during adolescence. Neurodevelopmental abnormalities then lead to the activation of pathological neural circuits which respond to environmental stressors, leading to the emergence of symptoms (Meltzer & Deutch, 1999). This hypothesis is supported by the finding that the MRI abnormalities are present at the onset of the illness, and progress very slowly if at all (Weinberger, 1995). A contrasting view is that the relationship between schizophrenia and brain volumes might be environmental in nature. Perinatal trauma has been shown to be an important determinant of some brain structure anomalies in schizophrenia (Verdoux *et al.* 1997; Cannon *et al.* 2002*a*, *b*; McNeil *et al.* 2000; McDonald *et al.* 2002).

The aetiological significance of these brain structure changes in schizophrenia remains elusive, whether they are caused by overlapping genetic or environmental factors. The current study uses a twin design to address this question in relation to selected regions of interest (ROIs), namely whole brain, lateral and third ventricular and hippocampal volumes. Unlike previous studies we adopt a formal genetic model-fitting approach to obtain estimates of genetic and environmental correlation. We propose the use of an adjusted bivariate genetic model to account for selection. Before applying this model to our data, we tested by means of simulations whether this model produced unbiased estimates. In addition we explored the power of our sample to detect a genetic correlation between schizophrenia and brain volumes under a range of simulated models.

METHOD

Subject recruitment and clinical assessment

Subjects were recruited from local and national psychiatric services in the UK after Multi Centre Research Ethics Committee approval had been granted. Healthy comparison subjects were ascertained from a pool of controls obtained for previous studies conducted at the Institute of Psychiatry, from members of staff at the Bethlem and Maudsley Hospital Trust and via advertisements in the local press. Monozygotic (MZ) control twins were mainly recruited from the volunteer Institute of Psychiatry twin register. All subjects gave written informed consent to participate in the study after a full explanation of the study aims and procedures. Subjects were excluded if they had a neurological illness or systemic illness with neurological complications, a past history of significant head injury involving loss of consciousness for more than 1 minute, or substance or alcohol dependence in the 12 months prior to scanning. All subjects underwent an extensive psychiatric and cognitive assessment. Structured clinical interviews were carried out using the *Schedule for Affective Disorders and Schizophrenia – Lifetime Version* (Spitzer & Endicott, 1978*a*) from which RDC diagnoses (Spitzer *et al.* 1978*b*) were produced. Additional clinical information was gathered to allow DSM-III-R diagnoses to be made.

MRI measurement

Image acquisition, analysis and inter-rater reliabilities of the ROI, namely whole brain, third and lateral ventricles, and hippocampus, are reported in detail elsewhere (van Haren *et al.*

https://doi.org/10.1017/S0033291705005167 Published online by Cambridge University Press

2004). In short, the whole brain volume included the cortical and subcortical grey matter and white matter, excluding the cerebellum, brain stem and CSF. The lateral ventricles extend forward into the frontal lobe as the anterior horn. This anterior part is devoid of choroid plexus (excluded from the segmentation) and was bounded medially by the septum pellucidum. The posterior horn extended posteriorally into the occipital lobe.

The third ventricle was bounded by the anterior commissure, the fornix, the stria medullaris, the pineal body, the superior and inferior colliculi, the midbrain and mamillary body, the thalamus and hypothalamus. In the coronal plane, the hippocampus was measured from the first slice where the mamillary bodies were present until the first slice where the fornix was clearly visible. The inferior boundary was simply the white matter of the parahippocampal gyrus, and the superior boundary was defined by the alveus. The temporal horn of the lateral ventricle served as the lateral boundary and the medial edge of the temporal lobe as the medial boundary. The alveus was used to differentiate the hippocampal head from the amygdala.

Variance components should be judged in combination with the reliability of the trait, as high measurement error always reduces the heritability estimate (Falconer & Mackay, 1996). The inter-rater reliabilities were all well over 0·88. Brain volumes were height, age and sex regressed and standardized. Missing data for height were substituted by mean values for that group. Brain volumes were ordinalized into five equal classes to avoid problems of non-normality and to facilitate genetic model fitting with schizophrenia. The rationale for the number of categories is mainly for computational reasons: we were trying to find a balance between information loss and making threshold model fitting feasible.

Statistics

Polychoric correlations

For the statistical analyses, data of the different groups (concordant MZ ill twins, discordant MZ ill twins, discordant MZ well twins, control MZ twins, concordant ill siblings, discordant ill siblings, discordant well siblings, and normal control subjects) were combined into three groups: individuals, MZ twin pairs and sib pairs. We applied liability threshold models, which assume that risk is distributed normally and that the disorder occurs only when a certain threshold is exceeded. This means that both affected and unaffected subjects (per group) are assumed to be part of the same distribution of liability to the disorder.

Prior to genetic model-fitting, polychoric correlations between the underlying liabilities for schizophrenia (Sz) and each brain volume (BV) were estimated by model fitting of the three-group data. The correlations modelled were: cross-trait within-member (Sz_{m1}–BV_{m1}), same-trait cross-members in pairs of MZ twins or sibs (Sz_{m1}–Sz_{m2} or BV_{m1}–BV_{m2}), and cross-trait cross-member in pairs of MZ twins or sibs (Sz_{m1}–BV_{m2} or BV_{m1}–Sz_{m2}). To simplify the interpretation of the data, several constraints were imposed. The cross-trait within-member correlations were constrained to be equal across all individuals in the sample, yielding only one Sz–BV correlation. The cross-trait cross-member correlations were constrained to be equal within the MZ twin and sib group separately such that Sz_{m1}–BV_{m2} = Sz_{m2}–BV_{m1}. The thresholds for brain volumes were estimated for each group separately. While significant cross-trait within-member correlations imply common aetiological influences, the power to distinguish between different sources of variance causing the correlation is derived from the cross-trait cross-member correlations of the MZ pairs and sib pairs. Significant cross-trait cross-member correlations (e.g. Sz_{m1}–BV_{m2}) imply that these common aetiological influences are familial. Whether these familial influences are genetic or environmental in origin, is indicated by the MZ:Sib ratio of these correlations. A 2:1 ratio is indicative of additive genetic effects, whereas a 1:1 ratio suggests influences of common environment in inducing a correlation between the traits. Non-significant cross-trait cross-member correlations imply that the common aetiological influences on schizophrenia and brain volume are due to individual specific environment (E), not familial effects.

Genetic model fitting

The aim of this study is to examine whether the correlation between schizophrenia and brain volumes is due to genetic overlap (e.g. the same neurodevelopmental genes) or to common

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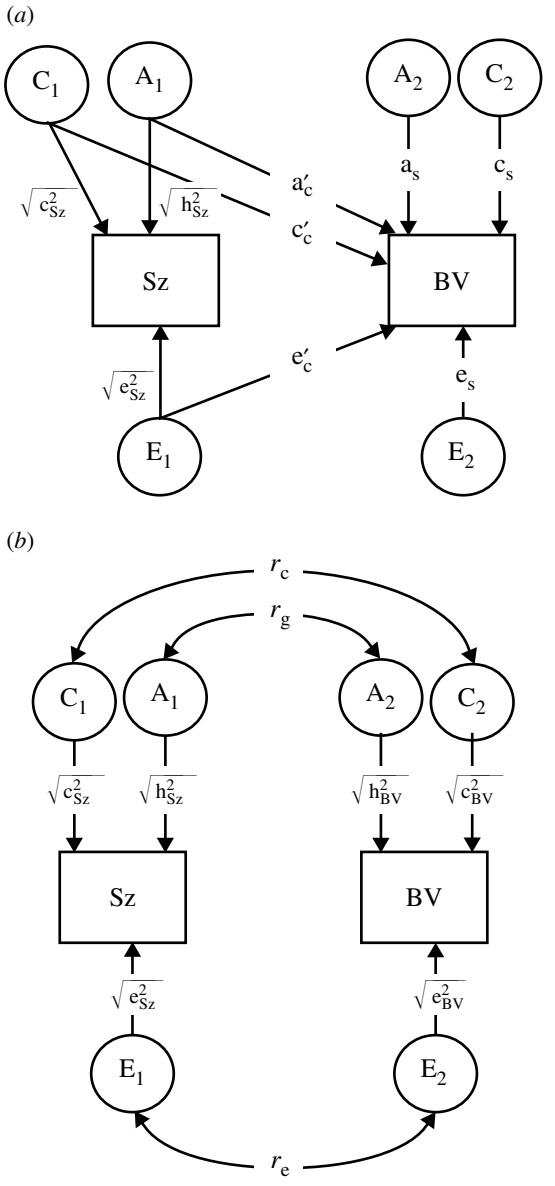


FIG. 1. Path diagram for the adjusted bivariate genetic model. (*a*) Cholesky decomposition. Parameters for schizophrenia (including the threshold) are fixed to postulated values, whereas for brain volume, genetic (a'_c, a_s) and environmental (c'_c, c_s, e'_c, e_s) paths and threshold are estimated (thresholds are not shown in this diagram). (*b*) The standardized solution of model (*a*): the correlated-factors model. Paths r_g, r_c, and r_e are the genetic, C and E correlation between the two variables. The total correlation $(r_{ph}) = (\sqrt{h^2_{sz}} * r_g * \sqrt{h^2_{BV}}) + (\sqrt{c^2_{Sz}} * r_c * \sqrt{c^2_{BV}}) + (\sqrt{e^2_{Sz}} * r_c * \sqrt{e^2_{BV}})$

individual-specific environmental effects (e.g. obstetric complications). The program Mx (Neale, 1999) was used for maximum-likelihood genetic model fitting to directly estimate model parameters from the observed data. The applied bivariate model is illustrated in Fig. 1*a*. Additive genetic effects (A), common environmental (C) and unique environmental effects (E) are specified such that factors A_1, C_1 and E_1 influence both schizophrenia (path a_c, c_c, e_c) and brain volume (path a'_c, c'_c, e'_c), inducing a familial covariance which is either genetic ($a_c * a'_c$) or environmental ($c_c * c'_c$) and an individual specific environmental covariance ($e_c * e'_c$). Factors A_2, C_2 and E_2 are specific to brain volume (a_s, c_s, e_s). If we do not wish to interpret a specific order of the variables, the solution of this decomposition can be standardized in a correlated-factors model (Fig. 1*b*), where, e.g. the paths from A_1 to schizophrenia and A_2 to brain volume are the square roots of their heritabilities, and where the correlational path between A_1 and A_2 is the genetic correlation (r_g). We can than calculate the part of the phenotypic correlation (r_{ph}), due to genetic effects by $(\sqrt{h^2_{sz}} * r_g * \sqrt{h^2_{BV}})$, the part due to C by $(\sqrt{c^2_{Sz}} * r_c * \sqrt{c^2_{BV}})$ and the part due to E by $(\sqrt{e^2_{Sz}} * r_e * \sqrt{e^2_{BV}})$.

There are two main methodological obstacles to model fitting in this study. The first is that schizophrenia is defined categorically, whereas the brain volumes are defined as continuous variables. To facilitate raw ordinal data analyses based on a liability threshold model, the brain volume data were categorized into an ordinal scale. A liability-threshold model can then be adopted for both schizophrenia and the brain volumes, in which the two liabilities are determined by potentially correlated genetic and environmental components. The second complication is that the data are from twin and sibling pairs selected for schizophrenia, rather than a random sample. Model fitting in selected samples usually requires an ascertainment correction. Since selection is through schizophrenia and blind to brain volumes, the required ascertainment correction will depend only on the model for schizophrenia. The need for this ascertainment correction is, therefore, obviated by fixing the model parameters for schizophrenia (heritability and prevalence) to constant values. However, since we do not know these values without error, we use three different models: the point estimates ($h^2 = 0{\cdot}81$, $c^2 = 0{\cdot}11$, $e^2 = 0{\cdot}08$) plus the lower and upper 95% confidence interval estimates ($h^2 = 0{\cdot}73$, $c^2 = 0{\cdot}19$, $e^2 = 0{\cdot}08$)

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and ($h^2 = 0{\cdot}90$, $c^2 = 0{\cdot}03$, $e^2 = 0{\cdot}07$) from a recent meta-analysis (Sullivan *et al*. 2003). In addition, we examined these three models for three different prevalence levels to give a lifetime risk of 0·05%, 0·75% and 1%. In contrast, the model parameters for brain structure (including liability thresholds), as well as the relationship with schizophrenia, are free parameters to be estimated from the data (see Fig. 1).

RESULTS

Sample characteristics

Twins

The sample consisted of 14 MZ twin pairs concordant for schizophrenia (mean age 34, range 19–48 years), 10 MZ pairs discordant for schizophrenia (mean age 36, range 21–64 years), and 17 MZ control pairs (mean age 36, range 19–57 years). All twin pairs were reared together. Approximately 30% were females and 10% were non-Caucasian. Zygosity was determined by structured physical similarity questionnaire and assessment of 13 highly polymorphic microsatellite markers. Birth order was determined in each of the discordant pairs; half of the probands ($n = 5$) were first-born. The members of each of the concordant and control twin pairs were randomly divided into two groups, with the restriction that the ratio of birth order was chosen to match the discordant group.

Sibling pairs and singletons

Twenty-two same-sex sibling pairs born within 10 years of each other and discordant for schizophrenia, three same-sex siblings concordant for schizophrenia, and 114 healthy control subjects without family history of psychotic illnesses in first- and second-degree relatives were also studied. Mean age of proband siblings was 33 years (range 24–50), mean age of control siblings was 32 years (range 17–56), mean age of control singletons was 36 years (range 19–65). These subjects are part of a larger family study that has been described in detail previously (McDonald *et al*. 2002). The discordant sibling pairs and healthy comparisons were matched with the twin pairs on sex, age, and hand preference. All sibling pairs and singletons were Caucasian, and ~30% of sib pairs and 50% of singletons were female. Detailed demographic information of the sample (apart from the three concordant sibling pairs and two healthy controls) has been reported elsewhere (van Haren *et al*. 2004).

Five of the non-schizophrenic co-twins in the discordant pairs satisfied criteria for a DSM-III-R Axis I diagnosis, namely major depressive disorder in remission. In the MZ control twin pairs, five subjects satisfied criteria for major depressive disorder in remission. In the discordant sibling pairs, four of the non-schizophrenic siblings satisfied the criteria for an Axis I disorder, namely major depressive disorder in remission. Finally, in the healthy control group, seven subjects satisfied criteria for a major depressive disorder in remission.

Table 1. *Estimates of model parameters when fitting fixed bivariate genetic model to simulated selected data*

	Simulated model				Estimated model parameters				
	h^2_{BV}	r_g	r_e	r_{ph}	h^2_{BV}	e^2_{BV}	r_g	r_e	r_{ph}
(1)	0·60	0·6	0·0	0·42	0·59	0·41	0·61	—	0·42
(2)	0·40	0·6	0·0	0·34	0·42	0·58	0·60	—	0·35
(3)	0·60	0·0	0·6	0·11	0·61	0·39	—	0·59	0·11
(4)	0·40	0·0	0·6	0·13	0·38	0·62	—	0·59	0·13
(5)	0·60	0·3	0·3	0·26	0·57	0·43	0·36	0·27	0·27
(6)	0·40	0·3	0·3	0·24	0·39	0·61	0·37	0·30	0·25

h^2_{BV}, heritability of brain volume; r_g, genetic correlation; r_e, unshared environmental correlation; r_{ph}, phenotypic correlation.

Simulated sample is current sample composition multiplied by 100. Model for schizophrenia is fixed at: $h^2 = 0{\cdot}81$, $c^2 = 0{\cdot}11$, $e^2 = 0{\cdot}08$.

Model-fitting results

Before analysing our data, we tested whether the proposed adjusted bivariate genetic model produced unbiased estimates in simulated data. Twin and sibling data generated under specified models were subjected to selection according to our sample composition (normal individuals, normal MZ pairs, concordant/discordant MZ pairs, and concordant/discordant sib pairs) (see Appendix). The model-fitting estimates of brain volume heritability and the genetic and the non-shared environmental correlation under different models were in close agreement with the simulated values and illustrate the validity of this approach (Table 1).

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Table 2. *Polychoric correlations within- and cross-member, and within- and cross-traits (with 95% CI) for schizophrenia and MRI brain volumes, estimated from combined analyses of individuals, MZ twins and siblings*

	Within-member cross-trait (Sz_{m1}–BV_{m1} = Sz_{m2} – BV_{m2})	Cross-member within-trait (BV_{m1}–BV_{m2})		Cross-member cross-trait (Sz_{m1}–BV_{m2} = Sz_{m2}–BV_{m1})	
Brain volumes	Whole sample (246 individuals)	MZ pairs (41 pairs)	Sib pairs (25 pairs)	MZ pairs (41 pairs)	Sib pairs (25 pairs)
Whole brain	−0·40 (−0·58 to −0·19)	0·93 (0·84 to 0·97)	0·30 (−0·14 to 0·65)	−0·38 (−0·55 to −0·16)	−0·20 (−0·49 to 0·05)
Hippocampus	−0·21 (−0·40 to 0·01)	0·55 (0·26 to 0·76)	0·39 (−0·03 to 0·70)	−0·12 (−0·31 to 0·09)	−0·08 (−0·33 to 0·18)
Third ventricle	0·28 (0·07 to 0·47)	0·69 (0·45 to 0·84)	0·51 (0·11 to 0·78)	0·24 (0·02 to 0·43)	0·08 (−0·18 to 0·34)
Lateral ventricle	0·08 (−0·13 to 0·29)	0·67 (0·42 to 0·78)	0·77 (0·50 to 0·91)	−0·02 (−0·23 to 0·20)	−0·11 (−0·34 to 0·13)

Sz, schizophrenia; BV, brain volume; subscript m, member.

The cross-member within-trait correlation for Sz (Sz_{m1}–Sz_{m2}) is constrained to be 0·92 in MZ pairs and 0·515 in sib pairs, and the threshold to give 1% prevalence. The within-member cross-trait correlations are constrained such that Sz_{m1}–BV_{m1} = Sz_{m2}–BV_{m2} and the cross-member cross-trait correlations are constrained such that Sz_{m1}–BV_{m2} = Sz_{m2}–BV_{m1}. Confidence intervals including zero indicate non-significance.

Polychoric correlations between schizophrenia and brain volumes for individuals, MZ twins and sib pairs were very similar across the nine tested models (three different fixed MZ and sib correlation models for schizophrenia × three different prevalences). In Table 2 the correlations are reported only for the model using the point estimates of the genetic model from the meta-analyses (Sullivan *et al.* 2003): $r_{MZ} = 0{\cdot}92$, $r_{DZ} = 0{\cdot}515$ and a prevalence of 1%. Results show that schizophrenia is associated with significantly smaller whole brain ($r = -0{\cdot}40$), and larger third ventricle volume ($r = 0{\cdot}28$). No significant correlation with hippocampal and lateral ventricle volume was observed. Also, the ratio of the twin and sib correlations suggest shared environmental effects in addition to genetic effects to explain hippocampal, third and lateral ventricle volumes.

Table 3 shows the results of the full ACE bivariate models fitted to the combined three-group schizophrenia and brain volume data (individuals, MZ and sib pairs). The estimated brain volume heritabilities (h^2), environmental variances (c^2, e^2), and the genetic (r_g) and environmental (r_e) correlation with schizophrenia are reported with 95% confidence intervals (CIs). In addition, the phenotypic correlations (r_{ph}) predicted by the ACE models are given. Since the difference in fit and estimates across prevalence levels was minimal, we report only the results across the three schizophrenia models for 1% prevalence. For whole brain volume C could be dropped without significant decline in fit, whereas A could not [$\Delta\chi^2$ (1 df) = 15·7, 24·7, 21·4; $p < 0{\cdot}01$ for models 1, 2 and 3 respectively]. The lowest estimated heritability was 88% (95% CI 0·46–0·97). For lateral ventricle volume there were no heritable effects: A could be dropped without significant decline in fit, whereas C could not [$\Delta\chi^2$ (1 df) = 8·8, 8·9, 8·8; $p < 0{\cdot}05$ for models 1, 2 and 3 respectively]. The lowest estimated effect of common environment was 67% (95% CI 0·24–0·81).

For hippocampal and third ventricle volumes the picture is less clear. Both show significant familial influences: the χ^2 differences when dropping the genetic and common environmental parameters at the same time exceeded the critical value of 9·5 for 4 df at the 0·05 level, but each set of two parameters for A and C can be dropped individually without significant decline in model fit (illustrated by 95% CI including a zero). This means that for hippocampus and lateral ventricles there was no power to distinguish between genetic and environmental sources of familial effects, also indicated by the twin and sib correlations (Table 2).

The genetic correlation between whole brain volume and schizophrenia was significant [$\Delta\chi^2$ (1 df) = 5·7, 7·4, 10·8; $p < 0{\cdot}05$ for models 1, 2 and 3 respectively]: $r_g = -0{\cdot}35$ (95% CI −0·65

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Table 3. *Estimates (with 95% CI) of full ACE genetic models for schizophrenia and MRI brain volumes (parameters for schizophrenia are fixed according to three different genetic models and a prevalence of 1%)*

	h^2_{BV}	c^2_{BV}	e^2_{BV}	r_g	r_c	r_e	r_{ph}
Whole brain							
Full ACE 1	0·88 (0·46/0·97)	0·04 (0/0·45)	0·08 (0·03/0·17)	−0·35 (−0·65/−0·08)	−1 (−1/1)	−0·34 (−0·93/0·34)	−0·39 (−0·56/−0·20)
Full ACE 2	0·90 (0·46/0·97)	0·03 (0/0·45)	0·07 (0·04/0·16)	−0·36 (−0·63/−0·11)	−1 (−1/1)	−0·33 (−0·92/0·35)	−0·39 (−0·55/−0·20)
Full ACE 3	0·91 (0·49/0·97)	0·01 (0/0·43)	0·08 (0·04/0·17)	−0·37 (−0·60/−0·16)	−1 (−1/1)	−0·33 (−0·95/0·36)	−0·38 (−0·54/−0·20)
Hippocampus							
Full ACE 1	0·36 (0/0·76)	0·20 (0/0·66)	0·44 (0·24/0·73)	−0·13 (−1/1)	−0·23 (−1/1)	−0·49 (−1/0·12)	−0·21 (−0·40/0)
Full ACE 2	0·38 (0/0·76)	0·18 (0/0·66)	0·44 (0·24/0·73)	−0·12 (−1/1)	−0·33 (−1/1)	−0·49 (−1/0·12)	−0·21 (−0·39/0)
Full ACE 3	0·32 (0/0·76)	0·23 (0/0·66)	0·45 (0·24/0·73)	−0·16 (−1/1)	−0·41 (−1/1)	−0·50 (−1/0·12)	−0·21 (−0·38/−0·01)
Third ventricle							
Full ACE 1	0·33 (0/0·83)	0·36 (0/0·74)	0·31 (0·16/0·54)	0·55 (−1/1)	−0·12 (−1/1)	0·31 (−0·26/0·75)	0·29 (0·07/0·47)
Full ACE 2	0·32 (0/0·83)	0·37 (0/0·74)	0·31 (0·16/0·54)	0·55 (−0·07/1)	−0·19 (−1/1)	0·30 (−0·26/0·76)	0·29 (0·07/0·47)
Full ACE 3	0·31 (0/0·83)	0·38 (0/0·74)	0·31 (0·16/0·54)	0·53 (−1/1)	−0·30 (−1/1)	0·31 (−0·25/0·76)	0·29 (0·10/0·47)
Lateral ventricles							
Full ACE 1	0·05 (0/0·49)	0·67 (0·24/0·81)	0·29 (0·17/0·46)	1 (−1/1)	−0·57 (−1/0·32)	0·63 (0·13/0·93)	0·08 (−0·13/0·29)
Full ACE 2	0·05 (0/0·46)	0·67 (0·27/0·81)	0·29 (0·17/0·46)	1 (−1/1)	−0·80 (−1/0·40)	0·63 (0·13/0·93)	0·08 (−0·11/0·29)
Full ACE 3	0·03 (0/0·15)	0·69 (0·32/0·81)	0·28 (0·17/0·46)	1 (−1/1)	−1 (−1/1)	0·66 (0·18/0·93)	0·12 (−0·05/0·29)

h^2, Heritability; r_g, r_c, r_e, genetic, shared and unshared environmental correlation; r_{ph}, total phenotypic correlation. Confidence intervals including zero indicate non-significance.
Fixed genetic models for schizophrenia used: (1) $h^2=0{\cdot}73$, $c^2=0{\cdot}19$, $e^2=0{\cdot}08$; (2) $h^2=0{\cdot}81$, $c^2=0{\cdot}11$, $e^2=0{\cdot}08$; (3) $h^2=0{\cdot}90$, $c^2=0{\cdot}03$, $e^2=0{\cdot}07$.

to −0·08) for model 1. Correlations due to shared and individual specific environment were not significant. For hippocampal volume the negative phenotypic correlation with schizophrenia was just significant for only the more powerful model 3, however there was no power to establish the sources of this correlation, since dropping r_g, r_c, and r_e individually all resulted in non-significant worsening of fit [$\Delta\chi^2$ (1 df) = 0·22, 0·04, 2·5; $p>0{\cdot}11$ respectively]. For third ventricular volume the positive phenotypic correlation with schizophrenia was significant across the three schizophrenia models, however, again, there was no power to establish the sources of this correlation, since dropping r_g, r_c, and r_e individually all resulted in non-significant worsening of fit [$\Delta\chi^2$ (1 df) = 2·5, 0·04, 1·05; $p>0{\cdot}11$ respectively].

For lateral ventricle volume an interesting pattern emerges. Although the individual specific environmental correlation is significant across all models for schizophrenia [$\Delta\chi^2$ (1 df) = 5·8, 5·9, 6·8; $p<0{\cdot}02$ respectively], due to significant C effects and a C correlation of opposite sign, the overall phenotypic correlation is non-significant across models ($r_{ph}=0{\cdot}08$–$0{\cdot}12$).

Power of current sample

Given the results described above, we explored the power of our current sample to detect two different hypotheses under different simulated genetic models (see Appendix): no heritable influences on brain volume (H_0: $h^2_{BV}=0$) and no genetic or environmental correlation with schizophrenia (H_0: $r_g=0$ or H_0: $r_e=0$). Table 4 gives the non-centrality parameter (NCP) ($\Delta\chi^2$ when the parameter of interest is dropped) and required sample sizes to reject the null hypotheses for each simulated model. To translate those sample sizes into the group structure of the actual sample, the number of pairs/individuals in each group was multiplied by the ratio N^*/N_{total}. For example, in model 1 the required sample size of 128 consists of $128/180=0{\cdot}72\times N$ of each group, which is 12 MZ control pairs, 10 MZ concordant pairs, seven MZ discordant pairs, two concordant affected sib pairs, 16 discordant sib pairs and 82 control individuals. The results show that the current sample, in terms of composition and size, is sufficient to detect phenotypic correlations of around 0·40, i.e. a relatively high genetic

https://doi.org/10.1017/S0033291705005167 Published online by Cambridge University Press

Table 4. *Results of power analyses of current sample*

	Simulated model				$\Delta\chi^2$ for H_0 (required sample size for 80% power)		
	h^2_{BV}	r_g	r_e	r_{ph}	H_0: $h^2_{BV}=0$	H_0: $r_e=0$	H_0: $r_g=0$
(1)	0·60	0·6	0·0	0·42	1094 (**129**)	—	1094 (**129**)
(2)	0·40	0·6	0·0	0·34	619 (**228**)	—	619 (**228**)
(3)	0·60	0·0	0·6	0·11	260 (**543**)	521 (**271**)	—
(4)	0·40	0·0	0·6	0·13	66 (**2141**)	574 (**246**)	—
(5)	0·60	0·3	0·3	0·26	317 (**446**)	106 (**651**)	217 (**1333**)
(6)	0·40	0·3	0·3	0·24	236 (**599**)	142 (**981**)	144 (**995**)

Simulated sample is current sample size multiplied by 100. Model for schizophrenia is $h^2=0{\cdot}81$, $c^2=0{\cdot}11$, $e^2=0{\cdot}08$. $\Delta\chi^2$, χ^2 difference when the tested H_0: $h^2_{BV}=0$ (null hypothesis of no heritability for brain volume); H_0: $r_g=0$ (H_0: $r_e=0$): null hypothesis of no genetic (environmental) correlation between schizophrenia and brain volume). The required sample size (N^*) is given in bold. For example 129 means: $129/180=0{\cdot}716\times N$ of each group, which is 12 MZ control pairs, 10 MZ concordant affected pairs, 7 MZ discordant pairs, 2 concordant affected sib pairs, 16 discordant sib pairs and 82 control individuals.

correlation of 0·6 with a brain volume heritability of 60% (model 1). Note that since the same correlation can be derived in different ways, there is also power to detect a lower genetic correlation with a higher brain volume heritability of, e.g. 80% or more (results not shown).

DISCUSSION

Structural brain abnormalities (MRI brain volumes) are among the most extensively studied endophenotypes in schizophrenia. The traditional statistical approach to demonstrate a genetic relationship with schizophrenia, is by comparison of means in family or twin studies. While abnormal mean volumes in the unaffected relatives (e.g. co-twins) of patients compared to healthy controls suggests a genetic relationship with schizophrenia (especially if this deficit is proportional to the genetic relationship to the proband when environmental influences are constant) a genetic correlation cannot be quantified.

In this paper we demonstrate that genetic model fitting to twin data is a more efficient approach, and additionally enables estimation of the heritability of the putative endophenotype. However, standard model-fitting methods are not directly applicable to such selected samples. We obviated the need for ascertainment correction by fixing (genetic) model parameters to values estimated by meta-analysis and the threshold to the population prevalence. Simulations showed that this model, when applied to selected data, returned unbiased heritability estimates for the endophenotype and the genetic and environmental correlations with schizophrenia. However, a limitation of this method is that the power will depend on the validity of the assumptions made about population prevalence and genetic model of the selection variable. We illustrated the adequateness of the adjusted genetic model by applying it to both simulated data and measured MRI brain volumes (whole brain, hippocampus, third and lateral ventricles) from our sample.

Our results are consistent with the results of a study in healthy subjects which reported a heritability for whole brain volume of around 80% (Baaré *et al.* 2001*b*). Our sample was underpowered to discriminate between genetic and shared environmental variance for hippocampal volume, consistent with other studies (Baaré *et al.* 2001*b*; Narr *et al.* 2002; van Erp *et al.* 2004). However, when hippocampal volume was corrected for total cortical grey-matter volume in unaffected twins (53 pairs) the AE model showed a non-significant minimally better fit than the CE model with a heritability estimate of 71% (van Erp *et al.* 2004). Our sample also lacked power to discriminate between genetic or shared environmental variance for third ventricle volume, which is in agreement with Sullivan *et al.* (2001). Lateral ventricle volume showed no evidence of genetic effects but significant effects of shared and non-shared environment, a replication of the results reported by Baaré *et al.* (2001*b*).

In terms of the relationship with schizophrenia, there was a significant genetic correlation ($r_g=-0{\cdot}36$) for whole brain volume, which is consistent with the comparison-of-means approach applied to the same sample (van Haren *et al.* 2004). Baaré *et al.* (2001*a*) reported a familial correlation, but could not distinguish between genetics and common environment.

We found no significant familial or environmental correlations for hippocampal volume and schizophrenia. This was surprising and

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disagrees with earlier reports that suggested a unique environmental correlation (McNeil *et al.* 2000; van Haren *et al.* 2004) or a genetic one (Narr *et al.* 2002; van Erp *et al.* 2004). Baaré *et al.* (2001*a*) detected evidence for a familial correlation but could not determine whether the relationship is genetic or common environmental in origin.

We found no significant familial or environmental correlations with schizophrenia and third ventricle volume due to the lower power associated with equal proportions of A, C and E. This is inconsistent with the results of the comparison-of-means approach used in the same sample (van Haren *et al.* 2004) and with McNeil *et al.* (2000) that suggested a unique environmental correlation.

The individual specific environmental correlation between lateral ventricle volume and schizophrenia was significant ($r_e = 0{\cdot}62$), but since C is significant and r_c negative, the total phenotypic relationship is non-significant. This illustrates the interesting patterns that can emerge when opposite sign relationships of different sources of covariance are detected by multivariate model-fitting. It is possible that common effects during pregnancy (e.g. substance abuse or medication exposure) cause a reduction in lateral ventricles which is later counteracted by individual specific effects such as fetal hypoxia leading to an increase in lateral ventricular volumes. However, only when results are based on adequately powered samples, is it worth speculating further about possible mechanisms. A significant environmental relationship with schizophrenia was also reported by other studies (McNeil *et al.* 2000; Baaré *et al.* 2001*a*) and using the comparison-of-means approach in the same sample (van Haren *et al.* 2004).

Given these results, we have explored the power of our sample to detect a genetic correlation between schizophrenia and brain volumes under a range of simulated models. The results showed that the current sample, in terms of its composition and size, is sufficient to reject the null hypothesis of no genetic effects on brain volumes for heritabilities of 60% or higher. There was sufficient power only to detect a relatively high genetic correlation of around 0·60 with brain volume heritability of around 60%, or smaller genetic correlations with heritabilities of 80% or more. Since other MRI twin studies in schizophrenic patients are hampered by dealing with comparable sample sizes, we have established an international consortium to pool samples across five European centres to overcome these power issues. MRI data of the combined sample will be processed according to a standard method which was calibrated for this purpose (Schnack *et al.* 2004). The genetic model-fitting analyses described above will then be applied to the combined sample, which will be adequately powered to: detect genetic correlations as small as 0·20 in the presence of moderate brain volume heritabilities (around 40%); to explore the effects of common environment; and to explore interaction effects including gender differences and environmental risk factors such as obstetric complications.

In studies with limited subject numbers, power studies will inform optimal study designs. We compared the power of a balanced design (15 twin pairs in each group of MZ and DZ control twins; MZ and DZ concordant twins, and MZ and DZ discordant twins) with alternative sample configurations in which certain twin classes were omitted (results available upon request). The configuration most comparable with the balanced design was that in which the informativeness of DZ concordant and DZ discordant pairs was substituted by increasing the number of (more readily available) normal control twins by a factor of 2. This knowledge can be particularly useful in the design stage of a study, when certain classes of subjects are known to be hard to ascertain.

Finally, application of the described adjusted genetic model and the results of the power studies generalize to other endophenotypes (e.g. ERPs) with comparable expected genetic models (h^2, r_g and r_e) and to other disorders with 'known' genetic and population prevalence parameters (e.g. ADHD, autism).

ACKNOWLEDGEMENTS

Collection of the data was supported by a grant from the Stanley Medical Research Institute. This research was sponsored by a travel grant (N. van Haren) of the Netherlands Organisation of Scientific Research (NWO: R56-465). Dr McDonald and Dr Picchioni were supported by the Wellcome Trust (064971).

https://doi.org/10.1017/S0033291705005167 Published online by Cambridge University Press

DECLARATION OF INTEREST

None.

APPENDIX

Data simulation

To investigate whether the proposed adjusted bivariate model produces the correct estimates, and to explore patterns in mean differences, data were simulated according to six different genetic models. Six datasets were simulated for each genetic model: (*a*) 11 400 normal individuals; (*b*) 1700 concordant normal MZ pairs; (*c*) 1400 concordant affected MZ pairs; (*d*) 1000 discordant MZ pairs; (*e*) 300 concordant sib pairs and (*f*) 2200 discordant sib pairs. The simulated numbers for each dataset are the group sizes of the actual study sample multiplied by a factor of 100. This is an alternative way of simulating 100 or more samples of the actual sample composition.

Standard normal variables were generated in which the phenotypic variance for schizophrenia was determined by additive genetic variation (A, 81%), shared (family) environment (C, 11%) and unique environment (E, 8%). For simplicity, only A and E effects were considered for brain volumes (either 60% or 40%). The genetic/environmental correlation between brain volume and schizophrenia varied according to different models chosen to generate phenotypic correlations between 0·11 and 0·42, typically observed in our data (column 1, Table 4). The standard normal schizophrenia variable was dichotomized at the z value of 2·34, and selected according to the affection status patterns of the different groups. Brain volumes were ordinalized in five categories (four thresholds, at z values $-1{\cdot}5$, $-0{\cdot}5$, 0·5, and 1·5).

Power analyses

We explored the power of the adjusted bivariate model to reject two different hypotheses under different simulated genetic models: the null model of no heritable influences on brain volume (H_0: $h^2_{BV}=0$) and the null model of no genetic or environmental correlation (H_0: $r_g=0$ or H_0: $r_e=0$). The required sample size (N^*) to reject the null hypotheses with 80% power is obtained by the formula:

$$N^* = \frac{\lambda}{\chi^2} * N_{\text{sample}},$$

where the critical NCP (λ) for 1 df (7·85) is divided by the observed NCP (χ^2 difference between a model in which, e.g. r_g is free *versus* $r_g=0$), multiplied by the actual sample size. N^* is further divided by the actual sample size (180) and multiplied by the N of each group, in order to get the required numbers of pairs for each group.

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