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Multivariate Genetic Analysis of Brain Structure in an Extended Twin Design

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The hunt for genes influencing behavior may be aided by the study of intermediate phenotypes for several reasons. First, intermediate phenotypes may be influenced by only a few genes, which facilitates their detection. Second, many intermediate phenotypes can be measured on a continuous quantitative scale and thus can be assessed in affected and unaffected individuals. Continuous measures increase the statistical power to detect genetic effects (Neale *et al.*, 1994), and allow studies to be designed to collect data from informative subjects such as extreme concordant or discordant pairs. Intermediate phenotypes for discrete traits, such as psychiatric disorders, can be neurotransmitter levels, brain function, or structure. In this paper we conduct a multivariate analysis of data from 111 twin pairs and 34 additional siblings on cerebellar volume, intracranial space, and body height. The analysis is carried out on the raw data and specifies a model for the mean and the covariance structure. Results suggest that cerebellar volume and intracranial space vary with age and sex. Brain volumes tend to decrease slightly with age, and males generally have a larger brain volume than females. The remaining phenotypic variance of cerebellar volume is largely genetic (88%). These genetic factors partly overlap with the genetic factors that explain variance in intracranial space and body height. The applied method is presented as a general approach for the analysis of intermediate phenotypes in which the effects of correlated variables on the observed scores are modeled through multivariate analysis.

KEY WORDS: Extended twin study; methodology; structural equation modeling; intermediate phenotype; MRI.

INTRODUCTION

The study of the genetics of human behavior has long focused on actual observable behavior, such as smoking, alcoholism, or intelligence (e.g., Maes *et al.*, 1999; Heath *et al.*, 1999; Bouchard and McGue, 1981). Although there is now clear evidence of genetic influences on these behaviors, it has often proven difficult to locate the particular genes that account for these influences (e.g., Petrill *et al.*, 1997; Flint, 1999). The genetic influence on observable behavior is the outcome of a complex interplay between several genes which each may have unique but small effects on the observed behavior. Kosslyn and Plomin (2000) suggested that to increase one's chances of finding the actual genes influencing behavior, it might be wiser to look for genes that are linked to more basic traits (i.e., more directly under the influence of DNA) than behavior. The more basic traits have become known as intermediate phenotypes or endophenotypes (Boomsma *et al.*, 1997; Lander, 1988; Kendler, 1999).

Indices of brain function are already widely used as intermediate phenotypes in the study of behavior. Changes in serotonin neurotransmission may affect mood

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and memory (e.g., Fink *et al.,* 1999), and electrical activity of the brain has been linked to alcoholism, sensation seeking, and cognition (e.g., Schukit, 1986; Rodriguez, 1999; Glass and Riding, 1999; Zuckerman, 1990). Electroencephalographic θ and α oscillations of the brain have been linked to memory performance (for a review see Klimesch, 1999) and the P300 evoked potential has been linked to general IQ (for a concise overview see Detterman, 1994). In the genetics of psychopathology, another main intermediate phenotype is brain structure. Indices of brain structure have been associated with schizophrenia (e.g., Lawrie and Abukmeil, 1998; McCarley *et al.,* 1999), mood disorders (e.g., Drevets *et al.,* 1997), and dementia (e.g., Kaye *et al.,* 1997). Although an obvious intermediate phenotype, human brain structure and volume have received little attention from geneticists. The few studies that have reported on the heritability of brain structure in humans generally report on very specific structures of the brain (e.g., Carmelli *et al.,* 1998; Steinmetz *et al.,* 1994) or have been conducted on small sample sizes (e.g., Bartley *et al.,* 1997). Thus, while many studies report genetic influences on behavior, and a number of studies link behavior to brain structure, there are virtually no studies that report on the genetic or environmental influences on brain structure.

In the light of future investigations of the genetic influences on brain structure or other intermediate phenotypes, we illustrate in this paper how Mx (Neale, 1997) can be used to analyze the genetic and environmental influences on a particular brain structure: the cerebellum. The cerebellum is one of the larger structures of the brain and lies posterior to the brain stem. It is thought to be involved in the coordination of movement and motor functioning (Ghez, 1991). Since the measurement of cerebellar volumes or other structures with magnetic resonance imaging (MRI) is costly, an approach is needed that minimizes the required number of subjects without affecting statistical power. A powerful design to study the genetic and environmental influences on a measured intermediate phenotype like brain structure is the extended twin design (Posthuma and Boomsma, 2000). In this design nontwin siblings are added to the classical twin design (as opposed to recruiting more twin families), which yields increased statistical power to detect genetic and shared environmental influences on a measured variable. Extended twin designs, however, provide data characterized by families of variable size, i.e., some families may include twins and one nontwin sibling, while other families may include twins and two or three nontwin siblings. Analyzing families of variable size means having to deal with the nuisance of having missing data and requires a statistical package which efficiently handles variable pedigree sizes.

Intermediate phenotypes are often correlated with other observed variables. For example, age and sex are known to affect cerebellar volume (see, e.g., Luft *et al.,* 1999; Raz *et al.,* 1998; Passe *et al.,* 1997). Also, cerebellar volume is expected to covary with body height and intracranial space. These two types of "confounders" need to be addressed differently. In nongenetic designs, it is common practice to regress out the effects of body height and intracranial space on brain volumes. However, in the hypothetical situation where half of the phenotypic variance in cerebellar volume is due to genetic factors which are shared with genetic factors that influence both intracranial space and body height, such a regression approach will lead to the conclusion that phenotypic variance in cerebellar volume is low. Applying a multivariate approach would correctly show the heritability of cerebellar volume.

In the present paper an approach is illustrated that deals with these two issues simultaneously: correction for linear effects of age and sex on multivariate observed scores (of cerebellar volume, body height, and intracranial space) in an analysis that allows estimation of genetic and environmental (co-)variation of these multivariate phenotypes. This analysis is embedded in an extended twin design to maximize the statistical power.

METHODOLOGY

A Linear Regression Model for Causal Effects on Observed Scores

Both age and sex are associated with body height, intracranial space, and cerebellar volume. In order to correct for these effects we employ a linear regression model for a continuous trait Y_j ($j = 1, \ldots, m$, where m is the number of phenotypes) with observed values $y = (y_{ij}, \ldots, y_{nj}$, where n is the total number of subjects).

In the subsequent analyses two explanatory variables (x_1 and x_2; age and sex, respectively) have causal effects on the observed individual scores of height, intracranial space, and cerebellar volume. All variables were multivariately normal distributed conditional on the values of age and sex except for body height in additional siblings. This was totally explained by one very tall male additional sibling. Inclusion of this individual did not influence the results presented in this paper.

The linear regression model for individual i (i =1, . . ., n) and trait j (1, . . ., m) is

$$\mu_{ij} = \beta 0_j + \beta 1_j age_i + \beta 2_j sex_i$$

where μ_{ij} is the expected value of individual i on variable j, age_i is the individual value of the first (age, in years) explanatory variable, and sex_i is the individual value of the second (sex; 0 denotes female, 1 denotes male) explanatory variable. $\beta 0_j$ is the intercept (grand mean) of variable j, $\beta 1_j$ is the regression estimate of age for variable Y_j, and $\beta 2_j$ is the deviation of males on variable Y_j.

Trivariate Analysis

Simultaneously with the linear correction for age and sex, the covariance of cerebellar volume with body height and intracranial space is modeled, using a triangular decomposition of the (co-)variance matrix of these traits (Neale and Cardon, 1992). With this decomposition it is possible to investigate whether the observed covariance between traits is due to a common set of genes and/or due to a common set of environmental influences. For example, diet habits may influence both body weight and cholesterol levels, yielding a phenotypic correlation caused by a common environmental factor.

We used a trivariate, triangular decomposition model including regression on the observed scores, in which all latent variances which are part of the variance decomposition model have been scaled to unity. This must be distinguished from the variances of the definition variables (age and sex) which are part of the regression model; these definition variables concern individual observed values and therefore have no variance (N = 1 for each individual).

The triangular variance decomposition model can easily be redefined as a model in which the common pathways are recalculated into correlations by following the general tracing rules of path analysis (Wright, 1934; Neale and Cardon, 1992, Chap. 13) and applying the general formula for calculating a correlation. If the coefficients from the paths of A1 (the first latent additive generic factor) to body height (B) and intracranial space (I) are denoted a1b and a1i, respectively, and the coefficient of the path from A2 (the second latent additive generic factor) to intracranial space is denoted a2i, the genetic correlation between body height and intracranial space [r_g (B, I)] is obtained as follows:

$$r_g(\mathrm{B,I}) = \mathrm{a1b} \times \mathrm{a1i} \,/\, [\mathrm{a1b}\sqrt{((\mathrm{a1i})^2 + (\mathrm{a2i})^2)}]$$

The (non-)shared environmental correlation is calculated analogously.

Handling Variable Pedigree Sizes

Extended twin designs provide data characterized by families of variable size. Such "incomplete" data can be analyzed in Mx (Neale, 1997) via full information maximum likelihood, which uses the observed data. To obtain a measure of how well the specified model for means and covariances fits the observed values, the raw data option in Mx calculates the negative log-likelihood (–LL) of the raw data for *each* pedigree (Lange *et al.*, 1976), as

$$-\mathrm{LL} = -k \log(2\pi) + \log|\Sigma| + (y_i - \mu_i)' \Sigma^{-1} (y_i - \mu_i)$$

where k (k = 1, . . ., p; p = number of family members times number of phenotypes) denotes the number of observed variables within a family (and can vary over families), $\Sigma(pxp)$ is the expected covariance matrix of family members, y_i (for i = 1, . . ., p) is the vector of observed scores, μ_i the column vector of the expected values of the variables, and $|\Sigma|$ and Σ^{-1} are the determinant and inverse of matrix Σ, respectively.

Combining the expression of the –LL for each pedigree with a linear model for the expected scores as outlined previously gives a new expression for the –LL:

$$-\mathrm{LL} = -k \log(2\pi) + \log|\Sigma| + (y_i - \beta 0 - \beta 1 x 1_i - \beta 2 x 2_i)' \Sigma^{-1} (y_i - \beta 0 - \beta 1 x 1_i - \beta 2 x 2_i)$$

Since the families are independent, their joint likelihood is simply the product of their individual likelihoods and the log of the joint likelihood is the sum of the log-likelihoods per family. Thus, summing the negative likelihoods (–LL's) of all families gives the –LL of the model. In Mx the –LL of the model is doubled because twice the difference between two models (2 [$-\mathrm{LL}_{full\ model} - (-\mathrm{LL}_{nested\ model})$]) is—under certain regularity conditions—asymptotically distributed as χ^2. Thus, two nested models (a nested model includes fewer parameters and does not introduce new parameters compared to the model under which it is nested), which provide –2LL's, may be subtracted to provide a $\Delta(-2\mathrm{LL})$ which has a χ^2 distribution. A high χ^2 against a low gain of degrees of freedom (Δdf) denotes a worse fit of the second, more restrictive model relative to the first model.

An example Mx job that can be used to conduct the trivariate analysis with a linear correction of age

and sex on the individual scores in a design with variable pedigree sizes is available at the Mx website, http://views.vcu.edu/mx/examples.html, in the brain section.

Subjects

Subjects were recruited from The Netherlands Twin Registry (Boomsma, 1998) (170 cases) and through the Utrecht Medical Centre Twin Sample (86 cases). All subjects underwent physical and psychological screening to exclude cases of pathology known to affect brain structure.

Subjects were 256 family members from a total of 111 twin families. In total, 34 siblings (aged 29.6 years; SD, 4.81 years; 15 female, 19 male), 32 MZ male twin pairs (aged 30.34 years; SD, 9.20 years), 17 DZ male twin pairs (aged 30.3 years; SD, 7.01 years), 21 MZ female twin pairs (aged 34.1 years; SD, 11.68 years), 20 DZ female twin pairs (aged 30.6 years; SD, 8.48 years), and 21 DZ opposite-sex twin pairs (aged 30.3 years; SD, 12.35 years) participated. Seventy-seven families consisted of a twin pair and 34 families consisted of a twin pair and one additional sibling.

Cerebellar volume and intracranial space were obtained by 1.5-T MRI as described by Baaré *et al.* (2000) and analyzed according to the method described by Staal *et al.* (2000) and Hulshoff Pol *et al.* (2000).

RESULTS

Descriptive Statistics

Significant correlations (corrected for the effects of age) were observed between body height and intracranial space (0.194 and 0.229 for males and females, respectively; see Table I) and between body height and cerebellar volume (0.280 and 0.194 for males and females, respectively). In addition, a substantial correlation of 0.593 for males and 0.575 for females was observed between intracranial space and cerebellar volume.

Twin- and sib-pair correlations, as given in Table II, suggest that cerebellar volume, as well as body height and intracranial space, is largely heritable. The low DZM correlation was due mainly to two DZM pairs with large intrapair differences. However, in these two pairs individual scores were in the normal range and there was no indication of environmental confounding, so they were included in the analyses.

Model Fitting

When using raw data, the fit (−2LL) of a model can merely provide information on how well a more parsimonious model fits the data relative to a more general model. To gain some insight into the fit of the ACE model, which is the basic model for nested models AE/CE and E, we report the −2LL of a saturated model. In this saturated model the means are modeled in a similar way as in the ACE models, while the variance/covariance structure is not modeled, and all variances and covariances in MZ and DZ twins are estimated.

First, univariate genetic models for height, intracranial space, and cerebellar volume were fitted to the data correcting for the effects of age and sex on the observed scores. The regression estimates of the linear regression models for the observed scores of body height, intracranial space, and cerebellar volume show that height, intracranial space, and cerebellar volume decrease with age in our sample and are larger in males

Table I. Means and Intercorrelations of Cerebellar Volume, Intacranial Space, and Height

	Mean	SD	Body height	Intracranial space
Body height (cm)				
Male	181.94	6.66	—	—
Female	168.50	6.55	—	—
Intracranial space (cm^3)				
Male	1504.10	107.01	0.194*	—
Female	1340.26	113.09	0.229*	—
Cerebellar volume (cm^3)				
Male	146.80	11.17	0.280**	0.593**
Female	133.56	12.15	0.194*	0.575**

* Significant at the 0.05 level.
** Significant at the 0.01 level.

Table II. Twin and Sibling Correlations by Zygosity[a]

	MZM (32)[b]	MZF (21)[b]	DZM (17)[b]	DZF (20)[b]	DOS (21)[b]	TSM (15 + 11)[c]	TSF (8 + 11)[c]	TSOS (11 + 12)[c]
Body height	0.78	0.92	0.61	0.64	0.47	0.70	0.31	0.15
Intracranial space	0.90	0.92	0.33	0.70	0.40	0.67	0.62	–0.07
Cerebellar volume	0.85	0.93	–0.06	0.78	0.27	0.66	0.77	–0.12

[a] MZM/MZF—monozygotic male/female; DZM/DZF/DOS—dizygotic male/female/opposite sex; TSM/TSF/TSOS—twin-sib pair male/female/opposite sex.
[b] Pairs.
[c] Twin-sib correlations are calculated as the mean correlation of all "first" twins with their nontwin sibling and all "second" twins with their nontwin sibling. The number of pairs denotes the number of first twins with siblings and the number of second twins with siblings. Please note that for TSM and TSF, in all families except DOS families, the nontwin sibling provides two correlations: one with the first twin and another one with the second twin.

Table III. Regression Estimates of the Linear Regression Model on the Means of Body Height, Intracranial Space, and Cerebellar Volume

	β_0 (grand mean)	β_1 (effect of age; age entered in years)	β_2 (deviation of males)
Body height (cm)	172.20	–0.11	13.16
Intracranial space (cm^3)	1345.63	–0.33	169.82
Cerebellar volume (cm^3)	140.94	–0.23	12.70

than in females (Table III). This decrease with age may also reflect a cohort effect in our sample.

From the univariate regression analyses the expected value for an individual can be calculated. For example, the expected cerebellar volume (cm^3) for a male subject aged 30 is $140.94 - (0.23 * 30) + 12.70 = 146.74\ cm^3$.

Simultaneous with the correction for the effects of age and sex, the remaining phenotypic variance was decomposed into sources of variance due to additive genetic factors, shared environmental factors, and nonshared environmental factors. Comparison of the fit of the variance decomposition models with the saturated model shows that the ACE model describes the data reasonably (body height and intracranial space) to well (cerebellar volume). The most parsimonious model of the variance decomposition models for all three variables was a model in which additive genetic influences and unique environmental influences contributed to the phenotypic variance, whereas the influence of common environmental factors was nonsignificant (Table IV). Table IV includes the estimates and 95% confidence intervals for A, C, and E as found in the full ACE model. As expected, the observed variance in body height is highly heritable; 72% (47–92%) of the total variance is explained by genetic factors in the full ACE model. The heritabilities of intracranial space and cerebellum are also high; estimates for sources of variance due to genetic factors are 65% (40–91%) and 81% (54–92%), respectively.

In the multivariate analysis the influence of common environmental factors was, again, not significantly different from zero (dropping C from the ACE model caused an increase in –2LL of 4.546 for a gain of 6 df's). The fit of the multivariate ACE model was reasonable compared to that of a saturated model (Δ–2LL, 138.681; Δdf,72).

The regression coefficients in the multivariate analysis (AE model) are slightly different from the regression weights as estimated in the three univariate analyses. Figure 1 shows the unstandardized estimates in the triangular variance decomposition model. The unique environmental correlations between body height and intracranial space and between body height and cerebellar volume were nonsignificantly different from zero ($\Delta df = 2$, Δ–2LL = 3.293) and were excluded from the models to which Table V refers. In Table V the genetic and unique environmental correlations (Table Va) and the standardized genetic contributions of body height and intracranial space to the total variance of cerebellar volume are given, as well as the unique genetic variance of cerebellar volume (Table Vb).

Table IV. Nested Sequence of Univariate ACE Models with Linear Regression of Age and Sex on the Observed Value of Body Height, Intracranial Space, and Cerebellar Volume Fitted to the Raw Data

	Saturated model		ACE model		CE model		AE model		Standardized estimates in full model (95% confidence interval)			Standardized estimates in best-fitting model (95% confidence interval)	
	−2LL	df	−2LL	df	−2LL	df	−2LL	df	A	C	E	A	E
Body height	1556.32	241	1574.34	250	1601.30	251	1575.69	251	72% (47–92)	17% (0–41)	11% (7–18)	89% (82–92)	11% (8–18)
Intracranial space	2991.761	241	3010.76	250	3037.76	251	3012.83	251	65% (40–91)	23% (0–47)	12% (8–19)	89% (12–92)	12% (8–19)
Cerebellar volume	1860.22	241	1863.76	250	1895.94	251	1863.94	251	81% (54–92)	7% (0–34)	12% (8–19)	89% (81–92)	12% (8–19)

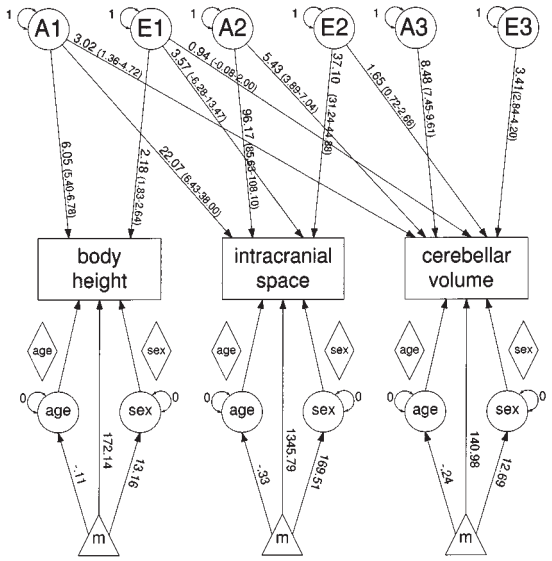

Fig. 1. Results of multivariate model fitting. The upper half shows the decomposition of the variance and covariance for body height, intracranial space, and cerebellar volume. Path coefficients are unstandardized; standardized estimates are given in Tables Va and b. The lower half represents the regression weights as estimated in the multivariate model, which may differ slightly from those estimated in the univariate analyses.

The genes that account for individual differences in body height also account to some extent for individual differences in both intracranial space and cerebellar volume (genetic correlations are 0.21 and 0.25, respectively). The genetic correlation of 0.57 between intracranial space and cerebellar volume indicates that some, but not all, of the genes that influence intracranial space are also important for cerebellar volume. Since the proportion of variance accounted for common environmental influences for each trait is relatively low, the common environmental correlation between intracranial space and cerebellar volume (0.44) can be misleading: although of medium size, it explains only a relatively small part of the total covariance between these two traits.

Six percent of the total variance in cerebellar volume is accounted for by genetic factors shared with body height, 24% is accounted for by genes that are shared with intracranial space, and 58% of the total variance in cerebellar volume is due to genetic factors that are unique to cerebellar volume.

DISCUSSION

Direct effects of age and sex on body height, intracranial space, and cerebellar volume were modeled

Table V

(a) Genetic (lower half) and unique environmental (upper half) correlations with 95% confidence intervals (in parentheses)

	Body height	Intracranial space	Cerebellar volume
Body height	—	n.s.	n.s.
Intracranial space	0.21 (0.06–0.36)	—	0.44 (0.21–0.63)
Cerebellar volume	0.25 (0.10–0.40)	0.57 (0.44–0.67)	—

(b) Standardized estimates in the multivariate approach of components of the genetic variance of cerebellar volume after correction for the effects of age and sex on the observed values

Cerebellar volume	Total genetic variance	Genetic variance due to genes that also influence body height	Genetic variance due to genes that also influence intracranial space	Remaining genetic variance unique to cerebellar volume
Estimate	88%	6%	24%	58%
95% confidence interval	81–92%	1–14%	14–36%	47–69%

simultaneously with a multivariate genetic model for the covariance between family members. For all three variables a slight decrease with age was found and a significant deviation for males, who were taller and had larger brain volumes than females. A trivariate genetic analysis was conducted on body height, intracranial space, and cerebellar volume, to dissect the pattern of covariance among these three variables and to determine the relative contributions of genetic and environmental influences to the remaining variance of each of these variables. For intracranial space and cerebellar volume, genetic factors accounted for 88% of the phenotypic variance. A large part of the genetic factors that are associated with cerebellar volume also controlled intracranial space (24%). Genetic factors that explain phenotypic variance in body height, however, accounted for only a small part of the genetic variation in both intracranial space and cerebellar volume. These findings suggest that studies using cerebellar volume as an intermediate phenotype will also need to consider the genetic covariance of cerebellar volume with intracranial space.

The causes of interindividual variation in human brain structure are largely unknown. This study shows that at least for one brain structure, cerebellar volume, interindividual differences are due largely to genetic variation between individuals. In mouse studies, several genes have already been implicated that influence development of the cerebellum. For example, Favor *et al.* (1996) showed that in mice, functioning of the *Pax2* locus, which has its counterpart in the human *PAX2* locus, is absolutely necessary for the normal development of the cerebellum. In addition, Millen *et al.* (1994) reported a reduction in cerebellar volume in mice due to dysfunctioning of the *En-2* locus.

Besides being of importance in its own right, a high heritability of human cerebellar volume in particular and brain structure in general may be of crucial importance in the study of causes of variation in complex behaviors. For example, correlations between brain size and psychometric IQ range between 0.38 and 0.45 [see Storfer (1999) for an overview of brain size–IQ relations], depending on which brain structure (i.e., gray matter volume, white matter volume, cerebral volume) is studied.

Quantitative intermediate phenotypes with high heritability are becoming more and more important in the field of behavioral genetics (e.g., Flint, 1999; Begleiter *et al.*, 1999; Boomsma *et al.*, 1997). These phenotypes are more "upstream," as Kosslyn and Plomin (2000) put it, and it is possible that they are influenced by a smaller number of genes, which could facilitate detection of these genes. In addition, quantitative intermediate phenotypes can also be obtained from nonaffected individuals. Thus, if a strong relationship between some brain structure and a psychiatric trait exists, such as the association between a reduction in prefrontal cortex volume and uni- and bipolar depression (Drevets *et al.*, 1997), it might be wiser to put a continuous index of prefrontal cortex volume in a time-consuming search for genes than to use a measure of uni- or bipolar depression.

Although a strong phenotypic relationship between the brain and the behavior is prerequisite, an equally important requirement for the intermediate phenotype to be of use in linkage studies is that it has a high genetic correlation with the behavior. Therefore, the intermediate phenotypes and the target behavior need to be analyzed in a multivariate design. Such a design must allow for the correction of covariates such as age and sex. Finally, since intermediate phenotypes require psychophysiological measurements, they are usually more complex and costly than behavioral measures obtained from observation, interview, or questionnaires, which makes it crucial to use an optimal statistical design. The present study shows that all three requirements for the genetic analyses of intermediate phenotypes (i.e., multivariate genetic analysis, correcting for linear effects on the mean, and optimal statistical power) can be handled in a single statistical approach using the Mx statistical package.

An additional advantage of the approach used in this paper is that it can easily be generalized to association analysis of quantitative trait loci (QTL). Measured covariates are not limited to sex and age but can also include polymorphic markers or candidate genes (e.g., Neale *et al.*, 2000) which can be modeled directly (Zhu *et al.*, 1999) or more sophisticatedly via within- and between-family effects (Fulker *et al.*, 1999; Sham *et al.*, 2000).

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