Twins and the fetal origins hypothesis: An application to growth data

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Summary

The Barker hypothesis states that size at birth is negatively associated with disease risk later in life. Numerous studies have tested and confirmed this hypothesis in singletons. Using twin (or sibling) data, several extensions of the Barker hypothesis may be considered:

1. Within pairs, is the smallest twin also the one with the highest disease risk later in life? Since twins (or siblings) come from the same family, this test controls for any shared family effects, such as maternal nutrition, parental education or socio-economic status.
2. A second extension compares associations of differences in size at birth with differences in disease risk in monozygotic (MZ) and dizygotic (DZ) twin pairs. If associations of difference scores are larger in DZ than in MZ twin pairs, this is taken as evidence that the association is mediated by genetic factors.
3. These two methods can be considered as alternative approaches to the full bivariate analysis of MZ and DZ twin data. Using a bivariate structural equation model, the correlation between two traits can be decomposed into genetic and environmental correlations.

We address some statistical questions regarding the relation of difference scores (within MZ and DZ pairs) and genetic and environmental correlations. We show that the comparison of associations between MZ and DZ difference scores does not necessarily provide clear-cut answers to the question of how the relation of size at birth and later outcome is mediated.

We present an empirical application to data on stature, birth weight and height assessed in a large sample of Dutch adult MZ and DZ twin pairs. There is a significant association between size at birth (both weight and length) and

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later stature. Within MZ and DZ twin pairs, the largest/heaviest twin at birth is the one who is tallest later in life. Using a bivariate structural equation model, we show that the association of birth length/weight and adult stature is explained by shared genes as well as by correlated common family and unique environmental influences.

Introduction

The "fetal origins" or "Barker" hypothesis states that body size at birth is negatively associated with cardiovascular disease risk later in life. Numerous studies have tested the Barker hypothesis in singletons, and evidence has accumulated that low birth weight is indeed associated with an increased risk of cardiovascular disease and that this association may be mediated by the association of low birth weight with blood pressure, insulin resistance, diabetes, plasma lipids, fibrinogen or hypothalamic-pituitary-adrenal axis activity (see, e.g., Phillips 2002 and the accompanying papers in the November issue of Trends in Endocrinology and Metabolism 2002).

One of the explanations for these associations, the "programming" hypothesis, states that poor intrauterine growth leads to metabolic changes that have adverse effects on cardiovascular risk and cardiovascular risk factors in later life. In testing this hypothesis, birth weight and /or height are often used as surrogate measures of fetal nutrition (Barker 1995, 1998; Barker et al. 1989; but see also Paneth and Susser 1995; Huxley et al. 2002).

If the Barker hypothesis is tested in genetically related subjects instead of unrelated cases (small size at birth) and controls, it becomes possible to conduct several explicit tests that might explain the association between size at birth and later outcome variables. The fetal origins hypothesis explains the association solely in terms of a causal mechanism, with poor prenatal growth leading to later adverse effects, although it is recognized that the strength of the association can be modified by genotype (Barker 2002, 2003) or later childhood growth (e.g., Eriksson et al. 2003).

Several alternative explanations of the Barker hypothesis may be considered if data from at least two offspring from the same family are available. The offspring can consist of siblings or twin pairs. In this paper we focus on the analysis of data from monozygotic (MZ) and dizygotic (DZ) twins, but the principles apply to sibling data as well. In a simple and straightforward analysis, one can test if the twin or sibling with the smallest size at birth is also the sibling with the highest disease risk later in life. Since twins and siblings come from the same family, this test controls for any shared family effects such as parental education, diet and socio-economic status (SES) and may control for lifestyle factors like nutrition and smoking during a twin pregnancy.

A second test, usually employed for twin data, is to look at the association of differences in size at birth within pairs with later differences in, for example, blood pressure, cholesterol levels, stature or BMI. This test is carried out separately for MZ and DZ twins. If the association of difference scores is larger in DZ twins than in MZ twins, this is taken as evidence that the relation of size at birth and later outcome is mediated by genetic factors. Differences between trait values of MZ twins can only be influenced by environmental factors that are unique to individuals. Differences between trait values of DZ twins are influenced both by environmental factors and by non-shared genetic factors. The association of difference scores for two traits such as size at birth and blood pressure is therefore expected to be larger in DZ twins if the part of the association is mediated by genetic factors.

The analyses of difference scores within pairs can be considered an alternative approach to a full bivariate analysis of MZ and DZ twin data, in which the complete distribution of scores for size at birth and for later outcome is modeled using covariance analysis or structural equation modeling. In this last approach the covariance, or correlation, between size at birth and later outcome is decomposed into genetic and environmental correlations, which may be tested for significance. An association of birth weight with, for example, adult stature can arise because environmental factors that influence birth weight also are of importance for stature later in life, or because genes that influence birth weight also influence measures later in life. Such effects are referred to as pleiotropic genetic effects.

In this chapter we focus on data obtained in MZ and DZ twins and address the question of what the relationship is between size at birth and stature later in life. Adult stature is reliably obtained by self-report, is determined mostly by genetic factors, and is associated with disease risk (McCarron et al. 2002; Silvertoinen et al. 2003). We examine the association within MZ and DZ pairs and determine the genetic and environmental correlations between size at birth and stature through genetic covariance analysis. We look at the usefulness of analyzing difference scores and present an algebraic derivation for their expectations.

Methods

Participants

The Netherlands Twin Register (NTR) has collected longitudinal data by mailed survey every two to three years in adolescent and adult twins and their family members. The twin families were recruited through city councils in 1990/1991. In 1990 all city councils in The Netherlands were asked for the names and addresses of twins aged between 13 and 20 years. Of the 720 city councils that were approached, 252 gave a positive response and supplied 4,036 addresses of

twin families. Between 1991 and 1993, additional addresses were obtained for 1,987 twin families. These included addresses from several of the larger cities. In the past 10 years, we have also recruited adult twins through city councils and have asked adult twins to register with the NTR in our yearly newsletter (Boomsma et al. 2002a).

Survey studies of health, lifestyle, personality and psychopathology have taken place in 1991, 1993, 1995, 1997 and 2000. A sixth survey is ongoing. A total of 8,219 twins participated in the first five surveys (3,226 twins participated once; 1,717 twice, 1,490 three times, 1,212 four times and 574 twins participated five times). Socioeconomic status (SES) for twins and siblings who are over 25 years (and most of whom have finished their education) is low in 22.1%, middle in 43.8% and high in 34.1% of the sample. Smoking behavior of twins and religious background of the participating families is comparable to that in the Dutch population (Boomsma et al. 1994, 1999).

For same-sex twin pairs, zygosity was determined from questions about physical similarity and confusion of the twins by family members, friends and strangers. For 804 same-sex twin pairs, information on zygosity based on DNA polymorphisms was also available. Agreement between zygosity diagnoses from survey and DNA data was 98%.

Measures

In all surveys, participants were asked about their current height and weight. The 1991 and 1993 surveys asked the mothers of twins, and in 1993 also the fathers, about the children's birth weight and height and gestational age. In 1993, 1995 and 2000, participants were asked about their own birth weight and height (but not gestational age). Extreme birth weights were checked by sending a response card to the participants and/or by phoning them. These participants were asked again for their birth weight and also were asked to indicate the source of the information, e.g., parents or hospital records.

For the definition of birth weight (BW), the following algorithm was used: if BW for twins was consistent across longitudinal surveys and consistent with parental report, this value was taken. If BW was not consistent over time, we took the values from the response cards when individuals indicated they checked BW with official records or with parents. Finally, if twins and parents gave different answers, but answers were consistent over time, we took BW as reported by the parents when within normal range (800-4500 grams); if not, we used the twin data. Similarly, for birth length the consistency across time and across parents and twins was checked. When twin data did not agree with the report of the parents, we used parental reports.

In this paper, we look at stature after age 20 years as the outcome variable in later life. Body height was obtained from all surveys between 1991 and 2000.

Table 1. Descriptive statistics for gestational age, year of birth, birth weight, birth length, and height for mono- and dizygotic twins (separately for males and females).

		GA	BYR	BW1	BW2	BL1	BL2	Height1	Height2
MZM	Mean	36.1	1974.2	2555.8	2509.2	47.8	47.2	182.9	182.5
	SD	3.15	3.10	543.73	547.94	3.00	2.99	7.77	7.24
	N	252	252	230	230	157	154	237	235
DZM	Mean	36.7	1973.9	2681.9	2626.4	47.9	48.2	183.2	183.2
	SD	2.92	2.93	529.31	564.65	2.81	3.03	6.70	6.67
	N	213	213	196	187	131	126	200	191
MZF	Mean	36.5	1974.4	2454.8	2425.1	46.8	46.9	169.5	169.4
	SD	2.99	2.92	524.44	502.23	2.93	2.93	6.00	6.09
	N	384	384	351	355	261	257	358	363
DZF	Mean	36.6	1973.8	2599.1	2464.3	47.4	47.2	170.7	170.0
	SD	3.19	3.09	521.38	542.16	2.78	3.13	6.29	6.76
	N	278	278	252	253	164	173	256	258
DZMF	Mean	36.7	1974.3	2699.4	2555.4	48.4	47.6	184.4	171.0
	SD	3.03	3.19	554.87	556.68	2.68	3.03	7.05	6.62
	N	235	235	190	216	129	148	193	224
DZFM	Mean	36.8	1973.9	2586.6	2665.4	47.5	48.2	171.3	183.4
	SD	3.17	2.90	511.12	560.54	2.88	2.78	6.23	7.32
	N	219	219	203	173	124	107	206	177

GA, gestational age; BYR, year of birth of twins; BW1 and BW 2, birth weight for oldest (firstborn) and youngest twin; BL1 and BL2, birth lengths; Height1 and Height 2, body height between ages 20 and 40 years; MZ, monozygotic: DZ, dizygotic; F, females; M, males; N, number of pairs for GA and BYR and number of individuals for the other traits.

Height data were only included when height was obtained at 20 years or older. Most individuals completed more than one survey. Differences in height across the questionnaires were checked and height data were discarded when there was no consistency across surveys and when differences were larger than 5 cm (for 44 of 5,994 twins). For a number of participants, height was also measured during experimental protocols. When measured height was available for age 20 years or older, this value was used in the analyses instead of self-reported height (793 of 5,994 twins). The correlation between self-reported and measured height was 0.93 (Silventoinen et al. 2003). Height data after age 20 years were available for 5,950 twins (3,406 pairs; 2,544 complete and 862 incomplete pairs).

There were 4,451 twins for whom gestational age was reported by the mother in 1991 and 1993 (2,215 complete and 21 incomplete pairs). The twins were born between 1966 and 1980, so that for, a large number of these twins, stature after

age 20 years had to come from surveys carried out after 1993. For these twins, height after age 20 years was available for 2,904 twins (from 1,320 complete and 264 incomplete pairs). For the analyses of birth length (reported by mother) and adult stature, there were 1,932 twins (from 836 complete and 260 incomplete pairs) and for the analysis of birth weight and stature, there were 2,862 twins (from 1,280 complete and 282 incomplete pairs) with data on these phenotypes. Table 1 gives the number of observations for each phenotype per sex by zygosity group.

Statistical analyses

As a first approach, we compared the stature of the twins with the lowest birth weight or length within each pair with the stature of their co-twins (who had the highest birth weight or length).

Paired *t*- tests were used to test if these differences in later stature were significant (Spss 11). For these analyses, twin pairs had to be excluded when the birth weight or length of the twins within a pair was the same. Also, because of the known sex differences in both birth size and adult stature, data from opposite sex twin pairs (who are always dizygotic) were discarded.

We derived the expectations for the variances and covariances of the differences in birth size and stature within MZ and DZ twins in terms of genetic and environmental variances for these traits and obtained the expectation for the association of the traits in terms of genetic and environmental correlations between them.

Bivariate modeling of the covariance between size at birth and adult stature was carried out with a standard software package for covariance structure analysis (Mx; Neale et al. 2003).

The data from MZ and DZ twins were used to decompose the variance in birth size and stature into a contribution of the additive effects of many genes, common environmental influences that are shared by twins (such as effects of household, socioeconomic level, or diet) and unique environmental influences that are not shared by twins (such as illness, but also measurement errors). Genetic influences are correlated 1 in MZ and 0.5 in DZ twins. Common environmental effects are perfectly correlated in both MZ and DZ pairs. Unique environmental influences are uncorrelated in both types of twins. For a summary of the twin method, the various assumptions, and the plausibility of these assumptions see, for example, Kendler and Eaves (1986), Neale and Cardon (1992), Martin et al. (1997), Plomin et al. (2001), and Boomsma et al. (2002b).

Twin correlations for the different sex by zygosity groups (e.g., MZ female, DZ opposite sex pairs) give a first impression of the genetic and environmental influences on size at birth and stature. If the traits are influenced by genetic variation, we expect the correlations between members of MZ pairs to be larger

than the correlations between members of DZ pairs. If the DZ correlation is larger than half the MZ correlation, this is evidence that shared environment contributes to twin resemblance, in addition to shared genes.

The analysis is based on the general model: $P = G + C + E$; where P stands for the observed phenotype and G, C and E for the latent (unobserved) genetic, common and unique environmental influences, respectively. Common environment refers to those environmental influences that are shared by members from the same household and that make them resemble each other. Unique environment refers to all non-genetic factors (including measurement error) that are unique to an individual and cause differences between family members. We assume there is no interaction or correlation between genotype and environment.

The variance (V) of a univariate phenotype can be decomposed as: $V(P) = V(G) + V(C) + V(E)$, assuming that genotype and environment are uncorrelated. The proportions of genetic and environmental variance in the phenotype (i.e., V(G), V(C) and V(E) divided by V(P)) are often referred to as h^2 (heritability), c^2 and e^2.

In case of a bivariate phenotype, the correlation between traits, e.g., X and Y, can be decomposed as: $r(x,y) = h_x h_y\, r(g) + c_x c_y\, r(c) + e_x e_y\, r(e)$, where r(g), r(c) and r(e) represent the genetic and the two environmental correlations between X and Y. These correlations are weighted by the square roots of the heritabilities of X and Y and by the square roots of c^2 and e^2 to obtain the phenotypic correlation between X and Y.

To obtain estimates of genetic, common environmental, and unique environmental variances and correlations, the 4x4 variance-covariance matrix of birth size and stature for twin 1 and twin 2 was analyzed. A saturated bivariate model also known as a triangular decomposition (Neale and Cardon 1992) was fitted to the data. First, we fitted a model with additive genetic, common environmental and unique environmental influences (ACE model), including sex differences in these parameter estimates. Next, we tested if the genetic and environmental correlations between birth size and stature could be constrained to be equal for males and females. Finally, we tested whether these correlations were significantly different from zero. Likelihood-ratio tests were used to test the fit of a more constrained versus a less constrained model, with the degrees of freedom (df) for the test equal to the number of (linearly independent) equalities or the number of parameters constrained to be zero. To make optimal use of all available data, the analyses were performed on raw data using the maximum likelihood estimation procedure for raw data analysis in Mx. The individual values for birth weight, length and stature were corrected for the possible effects of sex, birth year and gestational age.

Results

There was an association between stature and birth weight for males (N = 1208) and females (N = 1634). In males, the correlation was 0.22 and the regression coefficient (cm per kg) was 2.8. In females, the correlation was 0.24 and the regression coefficient also was 2.8. There was an association between stature and birth length in both males (N = 804) and females (N = 1128). The correlation in males was 0.30 and the regression coefficient (per cm) was 0.74. In females, the correlation was 0.27 and the regression coefficient was 0.56. All these associations were statistically significant.

Correlations of gestational age and birth weight were 0.50 (N=1208) and 0.54 (N=1634) in males and females. Correlations of gestational age and birth length were 0.43 (N= 804) and 0.43 (N=1128) in males and females.

Table 1 gives descriptive statistics for gestational age (GA), birth year, birth length and weight and adult stature for MZ and DZ twins, separately for males and females. There were no differences in gestational age or year of birth between the six groups ($p > .10$). For the other variables, a 2-way ANOVA with sex and zygosity was carried out (separately for first- and second-born twins). There were significant differences between MZ and DZ twins for birth weight ($p > .000$, both in first- and second-born twins), for birth length ($p = .012/.000$ in first- and second-born twins) and stature ($p=.022/.085$), with DZ twins being taller and heavier. The differences between the sexes for all three traits were also significant, but there were no interactions between sex and zygosity.

Table 2 summarizes the differences within MZ and DZ same-sex twin pairs for birth length and weight and stature. For birth weight, a difference of around 300 grams in MZ pairs is associated with a 0.9 cm (males) and 0.8 cm (females) difference in adult stature. In the DZ pairs, there is a somewhat larger difference in birth weight within pairs, but only in DZ females is this associated with a larger difference in stature (1.7 cm) than was observed in the MZ pairs. For birth length, a difference of around 1.75 cm in MZ twins is associated with a difference in adult stature of around 0.8 cm. In DZ twins, the differences between pairs in birth length and adult stature are even larger: a nearly 2 cm difference in birth length is associated with a 1.8 (males) and 3.2 cm (females) difference in adult stature.

These simple t-tests for paired observations are straightforward and informative. They confirm that, within families, differences between twins in size at birth are associated with later differences in adult stature. This finding implies that the association between size at birth and adult stature cannot entirely be explained by differences in parental social economic status that influence both size at birth and adult height.

There are some disadvantages to this approach: data from opposite sex pairs are discarded and data from twin pairs in which both members have the same

Table 2A. Average values for birth weight (BW) and for height after age 20 in same-sex twins with the highest and smallest birth weight within a pair (pairs in which twins had equal birth weights were excluded).

	MZM	DZM	MZF	DZF
N (pairs)	197	166	309	215
BW max	2670	2833	2586	2727
BW min	2368	2466	2296	2350
Difference in BW	303	366	290	377
t (p)	16.11 (.00)	14.15 (.00)	18.62 (.00)	17.30 (.00)
Height for BW max	182.8	183.6	169.7	171.2
Height for BW min	181.9	182.9	168.9	169.5
Difference in height	0.9	0.7	0.8	1.7
t (p)	4.67 (.00)	1.29 (.2)	6.26 (.00)	3.81 (.00)

Table 2B. Average values for birth length (BL) and for height after age 20 in same-sex twins with the highest and smallest birth length within a pair (pairs in which twins had equal birth lengths were excluded).

	MZM	DZM	MZF	DZF
N (pairs)	99	87	158	108
BL max	48.0	49.1	47.6	48.2
BL min	46.2	47.0	45.9	46.3
Difference in BL	1.8	2.1	1.7	1.9
t (p)	13.64 (.00)	16.34 (.00)	17.26 (.00)	15.45 (.00)
Height for BL max	183.2	183.9	170.1	171.6
Height for BL min	182.5	182.1	169.2	168.4
Difference in height	0.7	1.8	0.9	3.2
t (p)	2.66 (.00)	2.45 (.02)	5.09 (.00)	5.71 (.00)

birth weight (or length) cannot be used (there were 70 pairs with the same birth weight and 226 with equal birth length).

The second, widely used approach to analyzing within-family effects uses regression analysis to analyze the relationship of differences within pairs in size at birth with differences in adult stature. We did not employ this approach for

Table 3. Expectations for variances difference scores within monozygotic (MZ) and dizygotic (DZ) twin pairs for traits Y and X, and for the covariance and regression of the difference scores. Expectations are expressed as functions of the environmental variances Var(E) and the genetic variances Var(G) of the two traits and the environmental and genetic covariances between them.

Statistic	MZ expectation	DZ expectation
Variance D(Y)	2Var(Ey)	Var(Gy) + 2Var(Ey)
Variance D(X)	2Var(Ex)	Var(Gx) + 2Var(Ex)
Covariance [D(Y),D(X)]	2Cov(Ex,Ey) = 2r(e)[SD(Ex)*SD(Ey)]	2Cov(Ex,Ey) + Cov(Gx,Gy) = 2r(e)[SD(Ex)*SD(Ey)] + r(g) [SD(Gx)*SD(Gy)]
Regression [D(Y),D(X)]	Cov(Ex,Ey) /Var (Ey)	2Cov(Ex,Ey) + Cov(Gx,Gy) / 2Var(Ey) + Var(Gy)

the analysis of the empirical data because it cannot provide clear-cut answers to the question of how the association between birth size and stature is mediated.

Table 3 provides a summary of the expectations in MZ and DZ twins (or siblings) of the variances of difference scores, their co-variances and regressions. These expectations are given in terms of the variance of the genetic and environmental factors that influence two phenotypes labeled X and Y and the genetic and environmental correlations between these two phenotypes. As can be seen, in MZ twin pairs, the variances and covariances of difference scores are a function of the unique environmental variances and the environmental correlation between the two traits X and Y. In DZ twins, the variances and covariances of difference scores are a function of both the genetic and the unique environmental variances and of the genetic and the environmental correlations between X and Y. These expectations make clear that the common practice of estimating and comparing the regressions of difference scores in MZ and DZ twin pairs is only of limited value if one wants to draw conclusions about the etiology of the association between X and Y.

For example, assume that the genetic and unique environmental variances for trait Y are both unity (Var (Gy) = Var (Ey) 1, i.e., a heritability of 50% for trait Y). Then, the regression of difference scores in MZ pairs equals cov (Ex,Ey), whereas in DZ pairs this regression equals 2/3 cov (Ex,Ey) + 1/3 cov (Gx, Gy). If the covariance of Ex and Ey is equal to the covariance of Gx and Gy, then the regression coefficients of MZ and DZ are the same, but there is still genetic mediation of the association between X and Y. To put this a bit more generally (regardless of the heritability of Y, as long as it is not zero): there always is a combination of values for the covariance of Ex and Ey and the covariance of Gx and Gy that leads to the same regression coefficients in MZ and DZ twins, whereas there still is genetic mediation of the association between X and Y. If Y is a heritable trait, but the covariance of Gx and Gy is zero, then the regression

in DZ is even smaller than in MZ twins. It seems safe to state, however, that if the regression in DZ twins is larger than that in MZ twins, this implies that pleiotropic genes mediate at least part of the association between X and Y.

The only approach that makes optimal use of all data and that leads to an unambiguous conclusion about the etiology of the association of two traits is a full genetic analysis of the bivariate distribution of X and Y (i.e., size at birth and stature). We used this approach to model the birth weight and stature and the birth length and stature data collected in Dutch MZ and DZ twin pairs. All data, including those from "incomplete pairs" (for whom, e.g., stature was available in one twin but not in the co-twin) were analyzed, using the raw data likelihood estimator in Mx, which handles data from studies in which part of the sample has missing data. The bivariate analysis supplies estimates of the heritabilities of birth weight, height and stature and of the correlations between genetic (G), common environmental (C) and unique environmental (E) factors that influence these traits.

Table 4 summarizes the correlations between twins for birth weight and length and adult height. For height, both in males and in females, the twin correlations are substantially higher in MZ pairs than in DZ twin pairs, indicating that most of the variance in height in the Dutch population is due to genetic factors. For birth weight and length, the twin correlations show another pattern: both MZ and DZ correlations are high, although the MZ correlations are still somewhat larger than the correlations in the DZ groups. This pattern indicates that common environmental factors will explain a large proportion of the variance in birth weight and length. As we have seen, gestational age is highly correlated with both variables, and because it is (nearly) always the same in twins from the same pair, this is one of the common environmental influences shared by twins.

Two series of bivariate genetic analyses were carried out: for birth weight and adult stature (Table 5A) and for birth length and stature (Table 5B). We began with fitting a full model with sex differences in all parameter estimates, i.e., in genetic and environmental variances and correlations. Next, we tested if the genetic and environmental correlations could be constrained to be equal for males and females. The non-significant decrease in likelihood in Tables 5A and 5B (twice the difference in likelihoods is distributed as χ^2) indicates that this constraint is allowed. Estimates for genetic, common environmental, and unique environmental correlations between birth weight and adult stature were 0.19, 1.00 and 0.36, respectively. The tests of constraining one, or all, of these correlations at zero are given in Table 5A and show that they are all significant. The proportions of variance in birth weight (corrected for gestational age and birth year) explained by genes, common environment and unique environment were 0.23/0.25, 0.38/0.41 and 0.39/0.34 (in males/females, respectively). For adult stature, these proportions were 0.90/0.90, 0.03/0.01 and 0.07/0.08. Applying the

Table 4. Twin correlations for stature in all participants aged 20-40 years and in a subsample with data on gestational age, and twin correlations for birth weight and height.

	Height	N	Height*	N	BW	N	BL	N
MZM	0.89	330	0.93	220	0.75	212	0.82	135
DZM	0.47	233	0.50	178	0.60	172	0.73	108
MZF	0.90	647	0.93	337	0.71	327	0.83	233
DZF	0.49	386	0.47	236	0.61	232	0.77	149
DZMF	0.47	270	0.49	182	0.68	176	0.76	118
DZFM	0.40	252	0.45	164	0.70	158	0.74	93

* Height in subsample with data on gestational age; BW/BL, birth weight/length; N, number of pairs; MZ, monozygotic; DZ, dizygotic; M, male; F; female

Table 5A. Bivariate analysis of birth weight and stature: log-likelihoods (LL) for the full and reduced models; tests of constraining genetic, common environmental and unique environmental correlations at zero.

	-2LL	# parameters	$\Delta\chi 2$	p
Full ACE model	33905.04	26	-	
Full model, no sex differences in correlations	33906.96	23	1.92	0.58
No r(c)	33912.74	22	7.70	0.00
No r(g)	33911.83	22	6.79	0.00
No r(e)	33982.21	22	77.17	0.00
No correlation	34109.26	20	204.22	0.00

Table 5B. Bivariate analysis of birth length and stature: log-likelihoods (LL) for the full and reduced models; tests of constraining genetic, common environmental and unique environmental correlations at zero.

	-2LL	# parameters	$\Delta\chi 2$	p
Full ACE model	26292.72	26	-	
Full model, no sex differences in correlations	26294.21	23	1.49	0.68
No r(c)	26299.31	22	6.59	0.01
No r(g)	26317.50	22	24.78	0.00
No r(e)	26356.79	22	64.07	0.00
No correlation	26517.17	20	224.45	0.00

Table 6. Decomposition of the correlations between birth weight (BW) and adult stature and between birth length (BL) and adult stature for males and females.

	Phenotypic correlation	Proportion explained by G	Proportion explained by C	Proportion explained by E
BW, males	0.22	0.34	0.43	0.23
BW, females	0.24	0.40	0.32	0.28
BL, males	0.30	0.68	0.16	0.16
BL, females	0.27	0.42	0.38	0.20

G, genetic influences; C, common environmental influences; E, unique environmental influences.

formula $[r(g)\ h_x h_y + r(c)\ c_x c_y + r(e)\ e_x e_y = r(x,y)]$ for the decomposition of the phenotypic correlation between birth weight and stature in males, we get: $0.19\sqrt{0.23}\sqrt{0.90} + 1.0\sqrt{0.38}\sqrt{0.03} + 0.39\sqrt{0.39}\sqrt{0.07} = 0.25$. Thus, in males, about 34% (i.e., $19\sqrt{0.23}\sqrt{0.90/0.25}$) of the observed correlation can be ascribed to genetic pleiotropy. About 43% of the correlation comes from correlated common environmental factors and 23% from correlated unique environmental factors. Table 6 gives a complete summary of the proportions of the correlation between birth weight and stature that are explained by correlated genetic and environmental influences.

The analyses for birth length and adult stature also showed the estimates for genetic, common, and unique environmental correlations to be significant (Table 5B). These correlations were estimated at 0.59, 0.40 and 0.41, respectively. The lower part of Table 6 summarizes the proportions of the observed correlations that are explained by these correlations.

Discussion

We saw correlations of 0.22-0.24 between birth weight and adult stature. Allison et al. (1995) observed a similar correlation of 0.24 for birth weight with adult height in a Minnesota twin sample aged 28 to 52 years. Allison et al. (1995) analyzed the intra-pair differences in MZ twins and found these differences to be associated with differences in adult height. They concluded that intrauterine environmental influences on birth weight have an enduring impact on adult height and that the intrauterine period is a critical period for the development of height. They did not model the birth weight–stature data in the DZ twin pairs from their sample. We also found differences in birth weight within MZ pairs to be associated with later differences in height and observed these differences in DZ twins as well. Bivariate analyses of the birth weight–stature relationship showed that 30 to 40% of the association was explained by correlated genetic

influences. Another 30 to 40% of the association was explained by correlated common environmental influences, and the remainder of the association was due to correlated unique environmental factors.

The correlation between birth length and adult stature was 0.30 in Dutch adult males and 0.27 in females. This relationship was mediated by pleiotropic genetic effects, as well as by correlated common and unique environmental influences.

It is important to note that both types of environmental factors could be interpreted as evidence for the fetal origins hypothesis, which explains the association in terms of a causal mechanism with poor prenatal growth leading to later adverse effects. Unique environmental factors could be related to intrauterine differences experienced by the heavy and light twin, respectively, of twin pairs who are discordant for birth weight. These factors would reflect the variance in intrauterine conditions within the same mother across fetuses. Common environmental influences could be related to intrauterine differences experienced by both twins from a pair. These factors would reflect the variance in intrauterine conditions across different mothers. The evidence from the bivariate analyses for the presence of pleiotropic genetic effects indicates that there are genetic polymorphisms that influence variation in size at birth and have an influence on later variation in stature. Polymorphisms in, for example, the genes for insulin-like growth factor-I (Vaessen et al. 2002), or the insulin-like growth factor-I receptor (Abuzzahab et al. 2003) that are associated with low birth weight might be examples of such pleiotropic genetic effects. Interestingly, the polymorphism in the gene for insulin-like growth factor-I has also been shown to influence susceptibility to diabetes and cardiovascular disease in later life.

Several large-scale epidemiological studies (e.g., Rich-Edwards et al. 1995; Yarnell et al. 1992; McCarron et al. 2002) have reported an inverse association between adult height and (cardiovascular) mortality in males and females from Europe and the United States. Similar associations were found in young-adult Japanese (Miura et al. 2001) and in a large sample of middle-aged South Korean male civil servants (Song et al. 2003). Miura et al. observed an inverse relation of birth weight and height with blood pressure and serum cholesterol levels. Song et al. observed an inverse association between height and all-cause mortality. There was little evidence, however, of associations with coronary heart disease. The strongest inverse associations were with death from stroke, respiratory disease, and external causes. These findings suggest that factors operating in early life, which influence fetal growth and height, also influence future cardiovascular health. The lack of an association between height and coronary heart disease in the Song et al. study suggests that additional factors may be required for short stature to translate into increased coronary heart disease risk.

In an earlier study of adolescent Dutch twins (Ijzerman et al. 2001a), we found that the association between size at birth and stature is also significant

during puberty. Most of the association in this earlier study was due to genetic factors influencing both size at birth and stature during puberty. The correlation between common environmental factors was not significant, which might be due to the fact that this adolescent sample was small (160 pairs).

Pietilainen et al. (2001, 2002) examined tracking of birth length and weight in a large sample of Finnish twin adolescents and when they were aged 16 and 18 years. Height in adolescence was predicted by both length and weight at birth. Bivariate analyses showed that the association ($r = 0.39$ in boys and 0.36 in girls) of birth length and stature at age 16 years was explained by genetic and environmental correlations. The estimates in males and females were, respectively, 0.36/0.32 for the genetic correlations, 0.74/0.75 for common environmental correlations and 0.25/0.40 for unique environmental correlations. In our data from Dutch adult twins, the genetic correlation of birth length and height was somewhat higher (0.59) and the correlations of common environmental factors somewhat lower (0.40) than in the Finnish adolescent sample. The estimate for the correlation between unique environmental factors was roughly the same (0.41). This finding may indicate that the etiology of the association shifts during puberty and young adulthood towards a larger genetic component. In fact, this is also the conclusion that is suggested by Pietilainen et al. (2002).

It has been questioned whether differences in birth size in twins are a suitable model for differences in birth weight in general, because intrauterine growth in twins is different from that in singletons (Doyle et al. 1999). However, associations of birth weight with, e.g., blood pressure (Ijzerman et al. 2000) or serum lipids (Ijzerman et al. 2001b) in twins are similar to those in singletons. Although intrauterine growth in twins may be different from that in singletons, the associations between birth weight and these traits in twins suggest that differences in birth weight in twins can be used as a model for differences in birth weight in singletons.

Using stature as an example, we have demonstrated how the predictions of the fetal origins hypothesis, i.e., that size at birth is negatively associated with disease risk later in life, can be tested in twins. Moreover, using a twin design offers the clear-cut advantage over the singleton case-control approach of yielding an answer to the question of which factors mediate the association of size at birth and later outcome variables. Analyzing the bivariate distribution of size at birth and later outcome variables gives estimates of the genetic pleiotropic, common and unique environmental contributions to the observed association. We have shown that the comparison of outcome variables within pairs discordant for size at birth gives an indication of whether common environmental effects are of importance. If the smallest twin at birth is also the one with the highest disease risk later in life, this suggests that household effects, or effects of socio-economic class cannot explain the entire association. This approach does not make full use of all data. Data from opposite-sex twins or

siblings are usually discarded as are data from pairs with the same birth weight or height.

Expectations for difference scores within MZ and DZ twin pairs (please note that the expectations for siblings are the same as those for DZ twin pairs) and for the regression of difference scores on each other indicate that this commonly used approach is of limited value. It cannot provide an unequivocal solution to the problem of how the association of birth size with later outcome variables is mediated. Only when the regression coefficients of differences in birth size on differences in later variables are larger in DZ than in MZ twin pairs is it safe to conclude that the association is mediated (in part) by genetic factors.

Estimates of the relative size of these genetic pleiotropic influences, and of the contribution of correlated common and unique environmental factors, may be obtained from the full bivariate analysis of the covariance structure in twin pairs.

Acknowledgments

Most of the data collection was funded by the Netherlands Organization for Scientific Research (NWO 575-25-006, 904-61-090, 904-61-193, 985-10-002). We also acknowledge the GenomEUtwin project supported by the European Union (Contract No. QLG2-CT-2002-01254).

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