Genetic and Environmental Influences on Adiponectin, Leptin, and BMI Among Adolescents in Taiwan: A Multivariate Twin/Sibling Analysis

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Circulating levels of leptin and adiponectin are closely associated with obesity. However, it is not known whether there are common shared genes or environment exerting influences on the levels of leptin, adiponectin, and BMI. We aimed to assess the relative contribution of genes and environment to adiponectin, leptin, and BMI individually as well as simultaneously to the three measures. Our subjects included a total of 228 twin/sibling pairs aged 12 to 18 (130 monozygotic twins, 68 dizygotic twins and 30 sibling pairs) were recruited from the middle schools. Multivariate analyses were applied to twin/sibling data using structural equation modeling. The results showed that intraclass correlations for adiponectin, leptin and BMI were higher in the MZ twins than those in the DZ/SP group. The relative contribution of genes to adiponectin (39%) was comparable to those of shared environment (40%). In contrast, leptin and BMI were influenced mostly by genes (74% and 89%, respectively). The multivariate genetic analyses showed that a latent factor underlying the three measures was identified, with BMI being equivalent to this latent factor. The BMI-dependent genetic factor explains only 15% and 34% of variation of adiponectin and leptin, respectively. These data indicate a differential contribution of genetic factors for the variation of adiponectin, leptin and BMI. More importantly, only a small portion of the genetic influences on adiponectin and leptin was attributed to BMI. Our findings provided more insight into the complex regulation of adiponectin and leptin in obesity.

Keywords: adiponectin, leptin, BMI, twins

Adipose tissue is not only a storage site for energy but also an active endocrine organ. It plays an important role in the regulation of many physiological processes, and dysregulation of these processes may lead to obesity and obesity-related metabolic disorders (Ahima, 2006; Rosen & Spiegelman, 2006). Adipose tissue releases many hormones, termed adipokines, involved in energy homeostasis and metabolic processes. Among them, leptin and adiponectin are most extensively studied (Rosen & Spiegelman, 2006). Both have been associated with body mass index (BMI), a surrogate measure of adiposity, but in opposite directions (Fleisch et al., 2007; Hung et al., 2006; Im et al., 2006; Monti et al., 2006). Existing data show that circulating leptin and adiponectin levels as well as BMI are highly intercorrelated (Fleisch et al., 2007; Hung et al., 2006; Im et al., 2006; Monti et al., 2006). However, most of previous family or twin studies have investigated the heritability of these traits individually. To date, there are only two studies employing bivariate twin analysis to observe a considerable genetic overlap between circulating leptin level and BMI (Kaprio et al., 2001; Wallace et al., 2004). Currently, it is not known whether there are shared genes or environment exerting influences simultaneously on the levels of leptin, adiponectin, and BMI.

Although the structure of leptin and adiponectin and its corresponding genetic locus have been well characterized (Klok et al., 2007; Yang & Chuang, 2006), their circulating levels are unlikely merely attributed to these genes. For example, the polymorphisms in *APM1* gene accounts for only a small proportion of

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adiponectin variance (~2–8%) (Menzaghi et al., 2007). Several studies also showed that genetic loci (Menzaghi et al., 2007; Rankinen et al., 2006) other than *LEP* and *APM1* genes were implicated to be linked to or associated with the circulating levels of leptin or adiponectin, and the main influences of these adipokine levels remain largely unknown. Previous studies in families reported significant genetic influences on the variation of the circulating levels of leptin (Bayoumi et al., 2007; Livshits et al., 2005) and adiponectin (Butte et al., 2005; Chuang et al., 2004; Comuzzie et al., 2001; Hicks et al., 2007; Lindsay et al., 2003; Miljkovic-Gacic et al., 2007). Nevertheless, heritability estimated from family studies might be confounded by the influences from shared environment and hence overestimate heritability. In this aspect, a twin study design can properly dissect the attribution of the influences from genes, shared environment, and nonshared environment. Subsequent twin studies in adults also indicate a large portion of the variance in circulating levels of leptin (Jenkins et al., 2001; Kaprio et al., 2001; Wallace et al., 2004) or adiponectin (Cesari et al., 2007; Storgaard et al., 2007) is due to genetic contribution. However, it is difficult to observe shared environmental influence in adults as it might decline later in life.

Since there is an increasing trend of obesity, type 2 diabetes and metabolic syndrome in children and adolescents worldwide (Imperatore, 2006; Yoon et al., 2006), it is particularly important to clarify the influences contributing to leptin or adiponectin levels in adolescents. So far, there are only few twin studies in youth. These studies also indicate a substantial role of genes for leptin (Li et al., 2005; Narkiewicz et al., 1999) or adiponectin (Storgaard et al., 2007). In the present study, we aimed to investigate whether the covariation of leptin, adiponectin, and BMI was attributable to common genetic or environmental factors in a sample of healthy adolescent twins/siblings in Taiwan. Multivariate twin analyses using structural equation modeling were employed to compare different models and estimate relevant genetic and environmental correlations.

Materials and Methods

Participants

A total of 228 twin/sibling pairs, consisting of 130 monozygotic (MZ) (68 females and 62 males), 68 dizygotic (DZ) twin pairs (30 females, 24 males and 14 opposite-sex DZ) and 30 sibling pairs (20 females and 10 males) were included in the current analyses. The participants were the twin or sibling pairs in a study denoted as Twin/Sibling Study of Insulin Resistance (TSIR) as described previously (Kuo et al., 2006). Briefly, a total of 192 twins and 6 triplets (only 2 members of each triplet with same-sex or non-missing data were included for this analysis), as well as their first-degree relatives, were recruited from middle schools in Taipei during the period of 2002 to 2005 without any requirement on subject's health status. Because monozygotic twins (MZ) are more common than dizygotic twins (DZ) in Taiwan (Chen et al., 1999), same sex sib-pair (age difference less than 2 years) families (N = 30) were also included to complement the number of DZ twins. All twins and sib-pairs were born and reared together in this study.

All participants had blood samples taken after an overnight fast and in a three hours protocol consisting of physiological and psychological assessments, and completed self-report questionnaires (Kuo et al., 2006). The twin zygosity assignment was determined by means of DNA genotyping using 5 highly polymorphic DNA microsatellite markers with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). For six twin pairs whose DNA samples were not available, their zygosity was determined on the basis of both parental and self-reports of physical similarity, which was demonstrated to have an accuracy rate as high as 96% (Chen et al., 1999). The study was approved by the institutional review board of National Taiwan University Hospital, and written informed consents were obtained from all adolescents and their parents after full explanation of the study.

Measurements

Body weight (to the nearest 0.1 kg) and height (to the nearest 0.1 cm) were measured with subjects in light clothing without shoes. Body mass index (BMI) was computed as weight in kilograms (kg) divided by height in meters (m) squared. Both plasma adiponectin and leptin levels were determined by means of a fasting blood sample and measured using radioimmunoassay (Linco Research Inc., St. Charles, MO). The intra- and inter-assay coefficients of variation were 9.3% and 15.3% for plasma adiponectin levels (Tsou et al., 2004), and 8.15% and 10.3% for leptin levels, respectively.

Statistical Analyses

Data Transformation and Pooling of Subgroups

We performed log transformations for adiponectin and leptin levels due to the skewed distribution. Measures of BMI, and log transformed adiponectin and leptin levels were winsorized (Barnett & Lewis, 1994) to 2.5 standard deviation (SD) from the mean (separately for each gender) because the outliers and gender differences of them were present. The procedures were conducted using SAS software version 9.1 (SAS Institute, Cary, NC). Finally, this resulted in the changes of 9, 5 and 10 observations in adiponectin, leptin and BMI, respectively and the normality of all variables (all skewness and kurtosis were within the range of –1 and 1).

To assess whether male twins could be pooled with female twins and whether DZ twins could be pooled with same-sex sib-pairs, assumptions about the equality in the means, variances, and covariances of the variables of interest between groups were examined using the statistical package Mx (http://www.vcu.

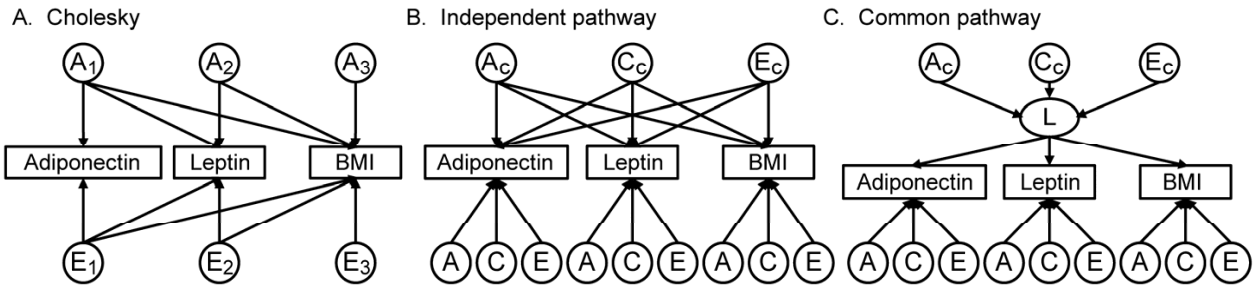

Figure 1

Path diagrams for multivariate analyses. A: The Cholesky model, in which additive genetic (A) effects are shown loading on the adiponectin, leptin and BMI. The shared (C, omitted in the figure) and nonshared environmental (E) effects would load similarly. B: The independent pathway model, in which a single genetic, shared and nonshared environmental factor loads on each of the three measures, as well as factors specific to each trait. C: The common pathway model, in which single genetic, shared and nonshared environmental factors load on the traits through a phenotypic latent variable (L), as well as factors specific to each trait.

edu/mx). First, we found no evidence for a significant effect of birth order, zygosity and gender on both the means and variances of the levels of BMI and log transformed adiponectin and leptin, as well as no substantial age effect on the means of these variables. Next, there were no differences in the covariances between male and female twins, between DZ twins and same-sex sib-pairs, and between same-sex DZ twins and opposite-sex DZ twins. These results indicated that we could combine MZ male with MZ female twins, and combine same-sex DZ twins with same-sex sib-pairs and opposite-sex DZ twins (designated as DZ/SP) in the subsequent analyses.

Genetic Modeling

Three types of correlation were estimated using Mx. The first one was the cross-trait correlation for each subject, that is, phenotypic correlation. The second one was the within-pair correlation for a specific trait, that is, intraclass correlations (ICCs). The third one was cross-twin cross-trait correlation. The contribution of genetic and environmental factors to the transformed adiponectin, leptin and BMI was determined using a univariate structural equation modeling approach. The total variation in each of the variables of interest was decomposed into additive genetic (A), common (or shared) environmental (C), dominant genetic (D) and nonshared environmental (E) variance (Neale & Cardon, 1992). The heritability (h^2) of each measure can be estimated as A / (A+C+E) or (A + D) / (A + D + E), which can be defined as the proportion of overall phenotypic variation that can be explained by genetic factors (additive genetic factors or both additive and dominant genetic factors).

In terms of multivariate analyses, the Cholesky model, independent pathway, and common pathway model were considered to assess whether there will be same or different genetic and environmental influences on the variables of interest simultaneously (Neale & Cardon, 1992). First, the Cholesky model permits systematic decomposition of the genetic and environmental covariance among the three measures of interest into independent factors. In this model, the first factor, for example the additive genetic factor (A_1), loads on all the variables; the second factor (A_2) loads on all except the first variable; the third factor (A_3) only loads on the last variable (A, Figure 1). Second, the independent pathway model specifies that each of the three common factors (Ac, Cc, and Ec) has its own path to each of the three variables. In addition, each of the variables is associated with three independent factors (A, C, and E) unique to the variable (B, Figure 1). Third, the common pathway model assumes that both genetic and environmental factors contribute to a latent factor (L), which has direct phenotypic paths to each of the variables. It also specifies that each phenotypic variable may be further influenced by specific environmental or genetic factors that are not shared with other variables (C, Figure 1).

All quantitative genetic model fittings were carried out on the raw data of individuals using normal theory maximum likelihood (Lange et al., 1976) implemented in the Mx, which permits twin or sibling pairs with partially incomplete data to be included. In general, a full ACE or ADE model was fitted and the goodness of fit of the full model was evaluated by comparing with a saturated model, in which the means are modeled in the same way as in the ACE or ADE model whereas the variance and covariance structure are not constrained for MZ twins and DZ/SP group. To find the most parsimonious model, a series of submodels with individual parameters removed from the full model were also examined. The fit of the full and its submodels was evaluated by means of the standard likelihood-ratio test, that is, the change in –2 log-likelihood (-2LL) along with in the difference in the degrees of freedom (df) between two models, which is asymptotically distributed as χ^2. A non-significant χ^2 value means that the more parsimonious model was preferred. Where models are not nested, the Akaike information criterion (AIC, calculated as of the χ^2 statistic minus twice the degrees of freedom) (Akaike, 1987) can be used for comparing the fit of two

Table 1

Descriptive Statistics of Study Subjects

	N	Age (year)		Adiponectin (mg/L)		Leptin (ng/ml)		BMI (kg/m^2)	
		Mean	(*SD*)	Mean	(*SD*)	Mean	(*SD*)	Mean	(*SD*)
MZF	136	14.0	(0.9)	10.7	(5.0)	14.3	(14.1)	19.7	(3.2)
MZM	124	14.2	(1.1)	8.6	(3.9)	5.4	(5.6)	20.6	(3.4)
DZF	60	14.3	(1.2)	10.4	(4.9)	13.4	(7.2)	19.7	(2.5)
DZM	48	14.1	(1.0)	8.3	(3.5)	4.1	(3.9)	19.9	(3.5)
OSF	14	13.6	(0.8)	9.1	(1.9)	14.1	(10.3)	19.6	(3.2)
OSM	14	13.6	(0.8)	7.2	(2.2)	4.2	(2.8)	19.5	(2.4)
SibF	40	14.2	(0.9)	7.9	(3.4)	12.8	(7.8)	20.0	(3.1)
SibM	20	14.4	(1.1)	7.4	(3.4)	5.3	(6.1)	20.7	(4.0)

Note: *N* = number of subjects, BMI = body mass index, *SD* = standard deviation, MZF = female monozygotic twin, MZM = male monozygotic twin, DZF = female dizygotic twin, MZM = male dizygotic twin, OSF = dizygotic opposite-sex twin (females), OSM = dizygotic opposite-sex twin (males), SibF = non-twin siblings (females), SibM = non-twin siblings (males).

models, with a lower value of AIC indicating a better fit of the model. Maximum likelihood parameter estimates and their 95% CIs for the separate genetic and environmental effects were estimated. For all analysis, a significance level of 0.01 was adopted.

Results

Background Characteristics

The subjects recruited were aged between 12 and 18 years (mean ± *SD*, 14.1 ± 1.1 years). The age distributions were similar among different zygosity or sibling groups (Table 1). The MZ group shows slightly higher levels of adiponectin and leptin than those of the other groups. In addition, the levels of adiponectin and leptin were higher in females than in males (Table 1). However, there was no significant difference between MZ twins and DZ/SP group with regard to mean and variance of these measures after data transformation.

Genetic and Environmental Influences on Adiponectin, Leptin and BMI

The MZ group had higher intraclass correlation than the DZ/SP group in terms of adiponectin, leptin, and BMI levels, indicating a significant but differential contribution of genetic factors for each of these measures (Table 2). Based on the univariate analyses, we found that the best fitting model for either leptin or BMI levels was AE model, while the full ACE model was the best model for adiponectin (Table 2), indicating a unique contribution of common environment for adiponectin. The parameter estimates in these best fitting models revealed that genetic factors, shared and nonshared environmental factors accounted for 39%, 40% and 21% of the total variance for adiponectin, respectively. For leptin, 74% of the variance was explained by genetic factors and 26% by nonshared environmental factors, while for BMI, the corresponding figures were 89% and 11%, respectively.

Genetic and Environment Influences on the Covariation of Adiponectin, Leptin and BMI

In phenotypic correlations, adiponectin levels were negatively correlated with leptin and BMI levels, and leptin levels were positively correlated with BMI levels (Table 3). The significant intercorrelations among the three measures indicate an existence of common etiological causes for the variations of adiponectin, leptin and BMI.

Table 2

The Intra-class Correlation and Univariate Structural Equation Modeling Parameters in the Study Sample (MZ = 130 pairs, DZ/SP = 98 pairs)

Phenotype	Twin correlation		Model fit						Parameter estimates in a best-fitting model		
			ACE/**ADE**		AE		CE				
	MZ (95% CI)	DZ/SP (95% CI)	−2LL	*df*	−2LL	*df*	−2LL	*df*	a^2 (95% CI)	c^2 (95% CI)	e^2 (95% CI)
Adiponectin	0.80 (0.73, 0.86)	0.57 (0.43, 0.69)	1079.9	447	1087.0	448	1092.1	448	0.39 (0.16, 0.67)	0.40 (0.12, 0.61)	0.21 (0.16, 0.28)
Leptin	0.77 (0.69, 0.83)	0.27 (0.08, 0.45)	**1146.6**	**446**	1146.9	447	1174.0	447	0.74 (0.66, 0.80)	—	0.26 (0.20, 0.34)
BMI	0.89 (0.85, 0.92)	0.33 (0.14, 0.49)	**1037.6**	**448**	1039.1	449	1124.1	449	0.89 (0.85, 0.92)	—	0.11 (0.08, 0.15)

Note: 95% CI = 95% confidence interval, −2LL = −2 log likelihood, *df* = degrees of freedom, a^2 = additive genetics, c^2 = shared environment, e^2 = nonshared environment. ADE models are shown in bold.

Table 3

Phenotypic and Cross-Twin Cross-Trait Correlation Coefficients

Variables	Correlation (95% CI)	Cross-twin cross-trait correlation (95% CI)	
		MZ (N = 130)	DZ/SP (N = 98)
Adiponectin vs. leptin	–0.32 (–0.42, –0.21)	–0.34 (–0.47, –0.19)	–0.14 (–0.29, 0.00)
Adiponectin vs. BMI	–0.44 (–0.53, –0.35)	–0.45 (–0.57, –0.32)	–0.11 (–0.26, –0.05)
Leptin vs. BMI	0.59 (0.51, 0.66)	0.56 (0.45, 0.66)	0.20 (0.04, 0.35)

Note: N = pairs; 95% CI = 95% confidence interval.

The cross-twin cross-trait correlations for the three measures between the MZ and DZ/SP groups were then examined (Table 3). For adiponectin versus leptin, the within pair cross-trait correlation was negatively greater for the MZ than for the DZ/SP groups. For adiponectin versus BMI, the within pair cross-trait correlation was also negatively greater for MZ than for DZ/SP groups. For leptin versus BMI, the within pair cross-trait correlation was positively greater for MZ than for DZ/SP groups. The consistent pattern of greater cross-twin cross-trait correlation for MZ than for DZ/SP group suggested that the shared etiological causes among the three measures were likely to have genetic components.

To further dissect the complex intercorrelations among adiponectin, leptin and BMI, three different trivariate genetic models, that is, Cholesky decomposition model, the independent pathway model, and the common pathway model, along with a series of submodels were compared for the goodness of fit and the corresponding statistics were summarized in Table 4. Starting with the full model for each of the trivariate models, all provided adequate explanation of the data as compared with the saturated model (all p values >.05). Since the ACE common pathway model (i.e., model 3, Table 4) had the lowest Akaike's information criterion (AIC) value among the three, this model was the preferable model from the point of view of parsimony and then was chosen for further simplification. By means of dropping one component (i.e., either A or C) each time for the latent factor or individual observed trait, it turned out that the following components could be dropped without leading to a significant worsening of the fit as compared with the full ACE common pathway model: C of the latent factor, C of leptin, and both A and C of BMI. Thus, BMI did not have unique genetic or shared environmental contributions other than those from the latent factor. In other words, all the variation of BMI could be attributed to the common pathway A_C or E_C via latent factor L. The final best fitting model explaining the relationship among adiponectin, leptin and BMI value was an AE common pathway model (i.e., model 12, Table 4), in which trait-specific A, C, and E were needed for adiponectin, trait-specific A and E were needed for

Table 4

Goodness of Fit Statistics for Multivariate Models

Models	–2LL	df	AIC	Compared to model	Δ-2LL	Δdf	p
0. Saturated model	2965.2	1308	349.2	—	—	—	—
1. Cholesky ACE	2999.1	1332	335.1	0	33.9	24	0.09
2. Independent pathway ACE	2999.9	1332	335.9	0	34.7	24	0.07
3. Common pathway ACE	3004.8	1336	332.8	0	39.6	28	0.07
Single reduction							
4. Drop A_{common}	3048.2	1337	374.2	3	43.4	1	0.00
5. Drop C_{common}	3004.8	1337	330.8	3	0.0	1	1.0
6. Drop A from adiponectin	3009.2	1337	335.2	3	4.4	1	0.04
7. Drop C from adiponectin	3015.4	1337	341.4	3	10.6	1	0.001
8. Drop A from leptin	3023.6	1337	349.6	3	18.8	1	0.00
9. Drop C from leptin	3004.8	1337	330.8	3	0.0	1	1.0
10. Drop A from BMI	3006.2	1337	332.2	3	1.4	1	0.23
11. Drop C from BMI	3004.8	1337	330.8	3	0.0	1	1.0
Multiple reduction							
12. Common pathway AE, with C dropped from leptin and ACE from BMI	3006.2	1341	324.2	3	1.4	5	0.92

Note: –2LL = –2 log-likelihood, df = degrees of freedom, AIC = Akaike's information criterion, Δ-2LL = difference in –2 log-likelihood, Δdf = difference in degrees of freedom, p value = probability value.

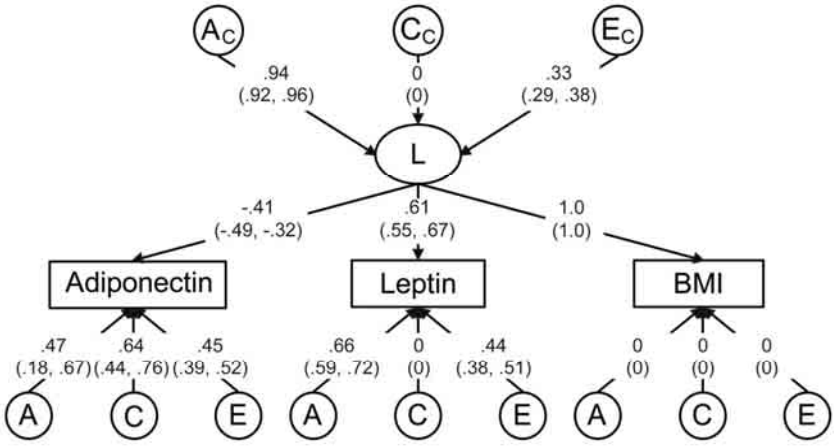

Figure 2

The AE Common pathway model and standardized path coefficients from latent genetic and environmental effects (circles) to the measured phenotypes (squares) through a phenotypic latent variable (L) for the data, as well as factors specific to each traits. Ac, Cc and Ec refer to common additive genetic, shared environmental and nonshared environmental effects. A, C and E refer to trait-specific additive genetic, shared environmental and nonshared environmental effects, respectively.

leptin, and all trait-specific components for BMI could be omitted ($\chi^2 = 1.42$, $p = .92$).

The final best fitting model and its corresponding estimates are displayed in Figure 2. We found that a latent factor L underlies the causes for variations of adiponectin, leptin and BMI. This latent factor L was mainly influenced by common genetic factor Ac (accounting for $0.94^2 = 89\%$ of the variance), whereas common nonshared environmental factor Ec only explained the remaining 11% (0.33^2) of the variance. Furthermore, the factor loading of BMI was estimated to be 1, indicating that this variable is isomorphic with the latent factor L. Further, the BMI (equivalent to the latent factor) accounted for a moderate portion of the variance in adiponectin ($-0.41^2 = 17\%$) and leptin ($0.61^2 = 38\%$). Accordingly, the proportion of variance of adiponectin explained by BMI-dependent common genetic effects could be estimated by multiplying the squared coefficients; that is, $(0.94^2) \times (-0.41^2) = 15\%$. The corresponding variance components of BMI-dependent and BMI-independent genetic and environmental factors for each individual trait are shown in Table 5. In general, the proportion of the variance explained by BMI-dependent genetic factors was much higher than that by BMI-dependent nonshared environmental factors for each trait. In addition to the BMI-dependent effects, there are BMI-independent effects on both adiponectin and leptin levels. For adiponectin, the BMI-independent shared environmental factors explained 41% of the variance, and the BMI-independent genetic and environmental effects further explained 22% and 20%, respectively. For leptin, the BMI-independent genetic factors explained 43% of the variance, and the trait-specific environmental factors explained the remaining 19% (Table 5).

Discussion

To our knowledge, this is the first twin study to examine the relative contribution of genetic and environmental factors in the associations among adiponectin, leptin, and BMI in a multivariate framework. Our results suggest that (a) there is a latent factor underlying all the three traits, with BMI being equivalent to this latent factor; (b) almost 90% of the contribution to the common latent factor (i.e., BMI is from genes); and (c) while the common genetic factor explains the majority of BMI's variation, the BMI-dependent genetic contribution to adiponectin (15%) and leptin (34%) was only modest. Previous studies have shown that Asian populations are likely to develop diabetes and obesity-related disorder at lower

Table 5

The Estimates of the Variance Components and Their 95% Confidence Intervals for Each Trait Under the Best Fitting Common Pathway Model

Variable	BMI-dependent		BMI-independent		
	a^2 (95% CI)	e^2 (95% CI)	a^2 (95% CI)	c^2 (95% CI)	e^2 (95% CI)
Adiponectin	15 (9, 21)	2 (1, 3)	22 (3, 44)	41 (20, 59)	20 (16, 27)
Leptin	34 (26, 41)	4 (3, 6)	43 (35, 51)	— —	19 (15, 26)

Note: a^2 = additive genetics, c^2 = shared environment, e^2 = nonshared environment, 95% CI = 95% confidence interval. All values are in percentages.

BMI and at younger age group than those of European descent (Yoon et al., 2006). Therefore, the current study may provide crucial insights into adolescent obesity and its related disorders in this vulnerable population at a young age period.

Previous studies reported a relatively high heritability of adiponectin among either adults (Chuang et al., 2004; Comuzzie et al., 2001; Hicks et al., 2007) or adolescents (Butte et al., 2005), except one of the study showed comparable low estimate of heritability (h^2 = 0.39) (Lindsay et al., 2003). In our present study, we found that the influences of shared environment and genes on plasma adiponectin levels were equally substantial at near 40%. The differences might be accounted for in part by that estimates derived from family studies inappropriately attributed all the familial aggregation to genetic factors and ignored the contribution from shared environmental factors. However, in two recent twin studies, high genetic influences for adiponectin levels, ranging from 58% to 88%, with no shared environmental factors were reported (Cesari et al., 2007; Storgaard et al., 2007). The lack of common environmental effect in previous studies may be due to limited power in detecting this effect in studies of small sample size. Taken together, our finding highlights the importance of shared environment, such as within-family dietary habits and physical activities, on circulating adiponectin levels and has implications for early prevention or intervention. Recent studies on the relationship between dietary habit and metabolic risk factors provide further support for the possible avenue through shared environment in reducing the risk of obesity or metabolic abnormalities. For example, in men with no history of cardiovascular disease, low adiponectin concentrations were found to be mediated through a high carbohydrate-rich diet (Pischon et al., 2005). Another example is that dietary habit of fast-food consumption predicted an increased risk of weight gain and insulin resistance 15 years later (Pereira et al., 2005). Our findings are in line with these studies and indicate that the effect of shared environment may occur as early as in adolescence. On the other hand, genetic factor plays a predominant role on both leptin levels and BMI. The high heritabilities of leptin and BMI estimated in this study are in line with those of previous reports (Narkiewicz et al., 1999; Silventoinen et al., 2007).

The multivariate analyses suggest that all the three traits are highly intercorrelated and there is a common latent factor underlying the covariations of these traits. Interestingly, BMI represents the core of the underlying latent factor that is influenced mainly by genes. In this model, BMI contributed a slight to moderate proportion of the total genetic variance to adiponectin and leptin levels, suggesting that there are additional BMI-independent contributions from genes and environment to variations of adiponectin and leptin levels. Adding support to our findings, linkage mapping for adiponectin levels were found on chromosome 3, 10 and 11 (Chuang et al., 2004; Comuzzie et al., 2001; Hicks et al., 2007; Lindsay et al., 2003; Pollin et al., 2005; Tejero et al., 2007), where linkage for BMI was implicated (Rankinen et al., 2006). In addition, several genetic association studies revealed that some single nucleotide polymorphisms (SNPs) were associated with both adiponectin levels and BMI (Rankinen et al., 2006; Yang & Chuang, 2006). Similarly, several genome scans demonstrated linkage of leptin to chromosome 2, 6 and 17 regions (Dai et al., 2007; Rankinen et al., 2006), where BMI was also found to be linked with (Rankinen et al., 2006). More convincingly, a recent bivariate linkage study revealed a susceptibility locus for both leptin and BMI in the chromosome 16q region (Dai et al., 2007). However, there are only limited studies to show covariation of circulating adiponectin and leptin levels. There is only one family study which showed common genetic factors were implicated for both adiponectin and leptin levels (genetic correlation = –0.3) (Butte et al., 2005). Previous studies suggested that both adiponectin and leptin levels were linked to chromosome 10 (Comuzzie et al., 2001; Hager et al., 1998). Future investigation is warranted to elucidate whether the same genes are responsible for the circulating levels of leptin and adiponectin.

In this study, we used BMI as a surrogate measure of adiposity instead of waist circumference. Although BMI was thought to have more limitations in assessing fat composition and its distribution than waist circumference, a recent longitudinal study revealed that there was no additional value of waist circumference in midchildhood, in addition to BMI, to identify those at increased risk of cardiovascular disease risk factors clustering in adolescence (Garnett et al., 2007). Another prospective study also showed that childhood BMI was predictive of coronary heart disease in adulthood (Baker et al., 2007). Nevertheless, waist circumference as an indicator of central adiposity in children and adolescent, in contrast to BMI as an indicator of total fat, has drawn more attention recently (Krebs et al., 2007). Further investigation is needed to evaluate the contribution of genes and environment underlying the relations of leptin and adiponectin to waist circumference.

Our results should be interpreted with caution. First, we incorporated sibling pairs with DZ twins from a genetic point of view. However, we had restricted the sibling pairs with age difference in 2 years. Moreover, the means and variances of the three traits of interest were equal for DZ twins and sibling pairs. Thus the impact of this potential bias might be minimal. Second, we did not explicitly control the influence of the pubertal status and timing on leptin levels in this study. Nevertheless, we found that 90% of female subjects were in late- or post-pubertal stage, and almost 80% of male subjects were in mid- or late-pubertal stage. After transforming the data of

leptin, adiponectin, and BMI to z scores separately for each gender, our preliminary analysis showed that pubertal status was not associated with any measures of interest. Third, despite gender differences in the levels of adiponectin and leptin, our latent structure model did not incorporate gender-specific parameters due to the limited sample size in this study. Finally, the major weakness of the twin method is viewed that the equal-environments assumption (EEA), that is, environmental influences do not differ for MZ and DZ twin pairs. The estimates of heritability may be inflated and estimates of shared environmental influences may be deflated if this assumption is violated. Although there is no direct evidence that EEA is not violated for levels of adiponectin, leptin, or BMI, previous studies on many behavioral traits presumably more vulnerable to this violation found that the validity of EEA was in fact supported (Evans & Martin, 2000).

In summary, the results of this twin study suggest that the contribution of genetic influences was moderate to substantial for the variation within each trait of adiponectin, leptin, and BMI, and very important for the covariation among them. These results provide empirical evidence to include BMI and leptin as well as adiponectin for future multivariate genetic analyses. Assessment of the relative contributions of genetic and shared environmental factors on adiponectin, leptin, and BMI may provide new insight in its physiological functions and the pathophysiology of obesity-related metabolic disorders. The findings may also help in the search for genes underlying variation and covariation in complex trait affected by plasma adiponectin concentrations, leptin levels and BMI.

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