Familial Resemblance for Plasma Leptin: Sample Homogeneity across Adiposity and Ethnic Groups

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Abstract

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Objective: Previous studies show a wide range in the percentage of variance in leptin levels attributable to genetic factors. These studies differ markedly with respect to ethnicity, study design, and statistical methodology. Therefore, the purpose of this study was to investigate heterogeneity hypotheses across ethnic groups and by adiposity level, using the same statistical methods.

Research Methods and Procedures: Samples included black vs. white (HERITAGE Family Study) and random vs. obese (Québec Family Study) individuals from 432 families (1432 individuals). Heritability for leptin, alternatively adjusted for age and sex and then for age, sex, and adiposity was estimated with the use of familial correlations. Heterogeneity in the magnitude of the familial resemblance between samples and the effect of adjusting for adiposity was explored.

Results: Heritability did not vary across samples stratified by adiposity level or ethnic group or across adjustment schemes. Maximal heritability, the percentage of additive phenotypic variability due to all familial sources, was 32%.

Discussion: Whereas leptin and adiposity were highly correlated within individuals, removing the effects of adiposity did not significantly alter the magnitude of the familial component for leptin. Moreover, this effect did not vary as a function of ethnicity (black vs. white) or adiposity level. Thus, no evidence for heterogeneity was detected. However, a comparison among previous studies raises questions concerning possible genetic heterogeneity in other ethnic groups in which complex interactions among leptin, adiposity, and diabetes status may be important.

Key words: heritability, sedentary, insulin, skinfolds, pleiotropy

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Introduction

Obesity is a major public health problem, is associated with morbid conditions such as type 2 diabetes and cardiovascular disease, and is certainly influenced by genetic determinants (1,2). One obesity locus originally identified in animal models is the mouse obese (*ob*) gene (3) that produces leptin. In humans, leptin is the product of the *LEP* gene (3). The gene is expressed in a few tissues, but circulating levels of leptin are primarily determined by adipose tissue (4,5). Blood leptin level is also associated with insulin sensitivity (6) and is higher in the obese than in leaner people (7) and higher in women than in men. Leptin has several functions, including inhibition of food intake and the maintenance of energy expenditure through a negative feedback loop through the sympathetic nervous system (8). Catecholamines and other hormones contribute to this loop. For example, insulin stimulates the secretion of leptin in the adipocytes of animals (9), although the effect in humans is

not as clear. There may be interacting factors such as the presence of impaired glucose tolerance or insulin resistance, or indirect trophic effects of insulin on adipocytes, leptin gene expression, or leptin secretion rather than acute regulation of leptin per se (8,10). Therefore, although the gene for leptin is known, its synthesis and release into circulation can be influenced by a myriad of factors, each of which can also have genetic causes.

Heritability provides an estimate of the percentage of variance in circulating leptin levels that can be accounted for by all of these contributing factors. Several previous studies examined these familial factors (11–19), and they reveal a substantial variability in the magnitude of the effect, from 0% to over 70%. Study designs (twin vs. family), statistical methods of analysis (correlations vs. variance components), population characteristics (ethnicity, adiposity level), and adjustments for correlated traits (e.g., adiposity) all differ across these reports. Therefore, whether the variability in these estimates is due to statistical/methodological differences or to possible heterogeneity in the underlying genetic causes is unclear. In particular, heritability differences across populations that differ with respect to adiposity level or ethnicity when methodology is held constant are consistent with the hypothesis of genetic heterogeneity.

In this study we address some of these issues using a familial correlation model that formally tests for heterogeneity across samples stratified by ethnicity and different rates of obesity. The Québec Family Study (QFS) includes different levels of adiposity and the HERITAGE Family Study (HERITAGE) consists of black and white families recruited from four clinical centers in North America. In addition to these basic sampling issues, the effects of adjusting leptin levels for adiposity on the estimates of heritability are examined. Finally, these results are put into perspective by comparing them to results from previous studies.

Research Methods and Procedures

Samples

The QFS (20) consists of families of French descent living within 80 km of Québec City. About one-half of the families were required to have one or more members with a body mass index (BMI) $\geq$32 kg/m^2 (obese sample), whereas the other half were randomly ascertained (random sample). The HERITAGE Family Study (21) consists of families selected for sedentary lifestyles. Sedentary was defined as those participants who, over the previous 6 months, participated in no regular vigorous physical activity more than once per week, i.e., any activity lasting 30 minutes or more and involving an energy expenditure of at least 7 units of energy expenditure (METS) for participants $\geq$50 years and of at least 8 METS for participants $<$50 years. With a few exceptions approved by a physician, the BMIs of the participants were $\leq$40 kg/m^2. Systolic/diastolic blood pressure (SBP/DBP) were $\leq$159/99 mm Hg. Of this sample, approximately two-thirds were white families and one-third were black. Moreover, approximately one-fourth of the sample was derived from each of four clinical centers (Bloomington, IN; Minneapolis, MN; Québec City, Québec; and Austin, TX).

Measures

In both studies, plasma leptin level (in nanograms per milliliter) was measured by radioimmunoassay (Linco, St. Charles, MO), which can detect human leptin with a sensitivity of 0.5 ng/mL. The coefficient of variation was 7% for repeated blinded assays. Leptin was log-transformed to reduce distributional kurtosis. Subcutaneous fat (in millimeters) was measured with a Harpenden skinfold caliper according to the International Biological Program (22) in the QFS and according to Lohman et al. (23) in the HERITAGE study. Six skinfolds were summed (SF6 = abdominal + subscapular + suprailiac + medial calf + triceps + biceps) for an overall measure of subcutaneous fat. The reproducibility of these measures was quite high (24).

Data Adjustments

Log-transformed leptin levels were adjusted for covariate effects with a stepwise multiple regression procedure, separately within each of 12 sex (male and female), age ($<$30 years, $\geq$30 and $<$50 years, $\geq$50 years), sample (QFS and HERITAGE) groups. Only terms that were significant at the 5% level were retained. The standardized residuals from these regressions were used in the genetic analyses. Two variables were constructed, one adjusted for age and the other for age and subcutaneous fat.

Familial Correlation Model

The univariate correlation model was based on four types of individuals [fathers (F), mothers (M), sons (S), and daughters (D)], yielding eight correlations within three familial classes [1 spouse (FM), 4 parent-offspring (FS, FD, MS, MD), and 3 sibling (SS, DD, SD)]. In the heterogeneity model, there were eight correlations for each subsample, with each correlation subscripted 1 through 4 for each of the 4 groups being analyzed (e.g., FM_1, FM_2, FM_3, and FM_4). The subscript order refers to QFS random (1) and obese (2) and HERITAGE black (3) and white (4). For testing across HERITAGE clinics, subscripts denote Indiana (1), Minnesota (2), Québec (3), and Texas (4).

The maximum-likelihood computer program SEGPATH (25) fit the model directly to the family data, assuming that phenotypes within a family followed a multivariate normal distribution. Null hypotheses were tested with the likelihood ratio test (LRT), which is the difference in likelihoods (-2 ln L) obtained under two nested models. The LRT is dis-

tributed as a χ^2 with *df* equal to the difference in the number of parameters estimated in the two models. The information criterion from Akaike (AIC; 26) (AIC = -2 ln L + twice the number of estimated parameters) was used to compare among non-nested models, and the "best" model was the one with the smallest AIC. Two series of tests were conducted. One series evaluated heterogeneity across subsamples and the other evaluated sex differences and the significance of the familial resemblance after homogeneous subsamples were combined. Means and variances were always estimated separately for each sample so that any heterogeneity would reflect differences in the correlations.

For testing subsample differences, a homogeneity model was estimated (i.e., parameters pooled across groups), and heterogeneity was tested separately for each class of relative pairs. For example, spouse correlations were estimated separately whereas the parent-offspring and sibling correlations were pooled across groups. A heterogeneity LRT was computed as the difference in the log likelihoods of these two models, with $df = [(N \text{ groups} - 1) \times N \text{ parameters}]$. The alpha level used to judge significance was $p < 0.05$. Because only homogeneous groups were pooled for the estimation of the familial effects described below, no ascertainment correction was considered for the QFS obese sample.

The correlations were tested for sex and generational differences and for significance in homogeneously pooled samples. In particular, tests included no sex differences in offspring (FS = FD, MS = MD, SS = DD = SD, $df = 4$), in offspring or parents (FS = FD = MS = MD, SS = DD = SD, $df = 5$), and no sex or generational difference (FS = FD = MS = MD = SS = DD = SD, $df = 6$). Significance was tested for all eight correlations simultaneously, as well as for familial class (spouse, parent-offspring, and sibling). Non-rejected models were combined to form the most parsimonious hypothesis, from which the maximal heritability (27) is computed.

Results

Sample Characteristics

Table 1 gives sample sizes, means, and SDs. Mean differences are noted in the legend. For most of the variables there is a general pattern of higher means in obese than random and higher in black than white, with some exceptions. Table 2 gives the results of the covariate adjustments for log-transformed leptin. As shown, SF6 is a strong predictor accounting for ~50% to 70% of the variance.

Heterogeneity

Heterogeneity testing results are in Table 3. In general, the spouse, parent-offspring, and sibling correlations were homogeneous among the four clinics of the HERITAGE Study and across the four subsamples for both leptin variables. Only the spouse correlation was heterogeneous for age- and SF6-adjusted leptin ($p = 0.0157$), and this was specific to the random vs. obese QFS comparison, i.e., FM_1 and FM_2 cannot be pooled, although $FM_3 = FM_4$. Familial analyses were then performed on the homogeneously pooled samples. For the age-adjusted leptin sample, all eight correlations were pooled across cohorts. For age- and SF6-adjusted leptin, all correlations except spouse are pooled [i.e., 10 correlations: 3 sample-specific spouse (FM_1, FM_2, and $FM_3 = FM_4$), 4 pooled-sample parent-offspring (FS, FD, MS, MD), and 3 pooled-sample sibling (SS, DD, SD)].

Familial Resemblance

The results for testing certain familial hypotheses are in Table 4. For age-adjusted leptin, there were no sex differences in offspring (model 2: $\chi^2_4 = 2.39$; $p = 0.6644$) or parents (model 3: $\chi^2_5 = 2.47$; $p = 0.7810$), and no sex or generational differences (model 4: $\chi^2_6 = 5.01$; $p = 0.5425$). The spouse (model 5: $\chi^2_1 = 5.40$; $p = 0.0203$), parent-offspring (model 6: $\chi^2_4 = 42.76$; $p < 0.0001$), and sibling (model 7: $\chi^2_3 = 16.51$; $p = 0.0009$) correlations were significant. Both the single correlation (model 8: $\chi^2_7 = 31.75$; $p < 0.0001$) and no-familial correlation hypotheses (model 9: $\chi^2_8 = 62.66$; $p < 0.0001$) were rejected.

For age- and SF6-adjusted leptin, there were no sex differences in parents or offspring (model 3), no spouse resemblance in QFS obese (model 5b), and no sibling correlations (model 7; $p = 0.0839$). The combined test of this hypothesis (models 3 + 5b + 7) fit by LRT ($p = 0.0702$), but the AIC (19.08) was larger than models 2 and 3. Therefore, the sibling correlation (with the smallest nonsignificant *p* value) was added back. The combined model of no sex differences in offspring or parents and no spouse correlation in the QFS obese sample (models 3 + 5b) fit the data by LRT ($p = 0.3246$) and AIC (14.96) and was chosen as the most parsimonious.

Table 4 also gives the most parsimonious familial correlations. Maximal heritability (27% to 32%) was consistent across analyses, considering the SEs. The significant spouse correlation suggests this effect was influenced in part by familial environmental factors.

Discussion

Several previous studies reported heritabilities for leptin using very different samples and statistical methods. Not surprisingly, the estimates varied from 0% to >70% (see Table 5). This variability may have resulted from methodological and statistical differences used across the studies, from heterogeneity in the underlying genetic effects across the different samples, or both. The current investigation of >1400 individuals from >400 QFS and HERITAGE families provided an opportunity to assess whether there is heterogeneity in the familial etiologies across very different

Table 1. Sample statistics

	QFS obese 268 individuals in 86 families			QFS random 377 individuals in 125 families			HERITAGE black 282 individuals in 121 families			HERITAGE white 504 individuals in 100 families		
Trait	***N***	**Mean**	**SD**	***N***	**Mean**	**SD**	***N***	**Mean**	**SD**	***N***	**Mean**	**SD**
Father												
Age	36	53.6	9.8	89	59.2	7.0*	25	50.9	7.3§	99	53.4	5.4*¶
BMI	36	32.6	7.2	88	26.9	3.8*	25	27.8	5.1†	98	28.3	4.4‡¶
FM	25	28.3	11.2	75	20.6	7.2*	21	20.8	6.8†	93	24.6	9.0*‡¶
SF6	36	134.1	52.6	83	91.5	29.9*	20	92.8	41.1†	88	113.7	36.8*‡¶
INS	36	110.4	63.7	81	65.6	51.0*	23	67.4	33.0†	96	79.3	64.6‡¶
Leptin	36	13.1	8.7	89	8.1	6.3*	25	7.3	5.6†	98	9.0	5.6*‡
Mother												
Age	43	51.0	8.6	102	60.5	12.0*	50	46.5	6.8†§**	88	52.1	5.1*¶**
BMI	41	34.5	8.4	101	25.5	4.2*,**	50	29.1	4.9†§	87	27.6	4.8*‡¶
FM	26	35.1	12.9**	71	22.3	7.9*	36	30.7	10.0†§**	80	27.2	10.6*‡¶**
SF6	37	204.7	73.7**	82	139.5	41.3*,**	38	168.2	44.5†§**	76	159.3	39.5‡¶**
INS	43	108.4	95.3	94	58.2	36.7*	46	81.8	75.2†§	87	63.3	30.6*‡**
Leptin	43	34.7	18.9**	102	22.5	13.6*,**	50	28.2	15.1†§**	88	24.0	15.6*‡**
Son												
Age	73	29.3	13.6††	93	31.4	10.9††	82	26.9	7.2§††	153	25.4	6.0*‡¶††
BMI	73	31.5	8.5	93	24.2	3.3*††	79	27.1	5.5†§	152	25.7	4.9*‡¶††
FM	54	28.7	19.3	92	13.2	7.2*††	76	20.3	12.6†§	141	17.2	11.0*‡¶††
SF6	71	149.2	71.6	93	72.9	34.0*††	74	89.6	47.4†§	146	93.0	41.2‡¶††
INS	73	116.0	69.6	83	47.0	31.4*††	73	76.2	53.7†§	147	66.5	40.3*‡¶††
Leptin	73	15.7	12.8	93	5.5	4.8*††	82	7.4	7.8†§	153	5.8	5.7*‡††
Daughter												
Age	117	31.3	12.4††	93	32.9	11.1††	125	28.0	7.7†§††	165	25.6	6.3*‡¶††
BMI	115	30.7	9.7††	92	23.5	3.1*,**††	124	27.7	6.7†§††	163	23.6	4.3*‡**††
FM	99	30.3	18.7††	90	16.3	6.1*,**††	106	26.9	13.3†§**††	163	17.9	9.5*‡¶††
SF6	115	179.3	80.6**††	92	103.6	37.3*,**††	98	134.8	49.7†§**††	162	118.4	39.4*‡¶**††
INS	117	101.9	73.7	84	46.6	23.9*††	114	77.0	60.3†§	162	58.1	25.7*‡¶**
Leptin	117	31.5	21.8**	93	16.3	10.6*,**††	125	27.5	19.5†§**	165	15.3	11.3*‡**††

QFS, Québec Family Study; HERITAGE, HERITAGE Family Study; BMI, body mass index; FM, fat mass; SF6, skinfold sum; INS, fasting insulin.

* Subsample differences within study (obese vs. random or black vs. white).

† Subsample differences across studies (obese vs. black).

‡ Subsample differences across studies (obese vs. white).

§ Subsample differences across studies (random vs. black);

¶ Subsample differences across studies (random vs. white).

** Sex differences within subsample and generation.

†† Generation differences within subsample and sex.

Note: Subsample mean BMIs are 32 (QFS obese), 25 (QFS random), 28 (HERITAGE black), and 26 (HERITAGE white).

subsamples (e.g., ethnicity and obesity status) when statistical and methodological considerations are held constant, i.e., similar family design, analytical model, and adjustment schemes. The current analyses suggest that the heritabilities were homogeneous across adiposity levels and black vs. white ethnic groups. However, whether these results can be

Table 2. Covariate adjustments

	QFS		HERITAGE	
Sex and age group	**Terms ($p < 0.05$)**	**% Variance**	**Terms ($p < 0.05$)**	**% Variance**
Age in years (men)				
<30	Age	8.7	Age	3.7
≥30 to <50	Age^2	18.1	None	—
≥50	None	—	None	—
Age (women)				
<30	Age^3	4.2	None	—
≥30 to <50	Age	3.3	None	—
≥50	None	—	None	—
Age, SF6 (men)				
≤30	SF6	48.8	SF6	65.7
≥30 to <50	SF6 Age^3	71.0	SF6	66.5
≥50	SF6 Age^3	51.7	SF6	52.8
Age, SF6 (women)				
<30	SF6	52.8	SF6	64.4
≥30 to <50	SF6	66.6	SF6 Age^3	52.7
≥50	SF6 Age^3	50.4	SF6	20.0

QFS, Québec Family Study; HERITAGE, HERITAGE Family Study; SF6, skinfold sum.

generalized to more extreme adiposity levels or other ethnic groups remains to be addressed.

The previous studies reported in Table 5 are arranged in descending order of magnitude of the heritability estimate. Twin studies generally yielded higher heritabilities than family studies. This frequently observed pattern can be explained in part by environmental and/or dominance deviation effects that are shared to a greater degree in twin that in non-twin relatives. Thus, in much of the following discussion, we will disregard the twin results except as specified.

In general, there is greater confidence in point estimates with smaller SEs (a direct function of the sample size), such as in the current study of >1400 individuals, the study by Luke et al. (16) of >1500 individuals, and the study by Kissebah et al. (19) of over 2000 individuals. In these three larger studies, the heritability for age- and sex-adjusted leptin ranged from 32% to 43%. For all nine family studies listed in Table 5, the range was larger (21% to 63%), although the weighted average (36%) was consistent with that for the three larger studies (35%). In comparing specific estimates, SEs were used to determine if the heritabilities were significantly different. Using this comparison, the report of Comuzzie et al. (12) of 63% heritability in Mexican Americans was significantly higher than in all of the remaining family studies, whereas the report by Walder et al. (17) of 21% in Pima Indians was significantly lower than the top three estimates of at least 42% (12,14,16). The only other significant difference was for nominally lower estimates in the current study (32%) compared with either the estimate by Luke et al. (16) in U.S. blacks and the estimate by Hsueh et al. (14) in Amish pedigrees, selected for obesity, of 42% to 43%. However, we note that our point estimate of 32% is the same as that of Kissebah et al. (19), which did not differ from the estimates by Luke et al. (16) and Hsueh et al. (14) because of a larger SE.

To summarize, there is consistent evidence for a larger estimate in the Mexican American sample (63%), a smaller estimate in the Pima Indians (21%), with the remaining estimates being moderate (average of ~35%) and primarily homogeneous. Thus, our current results suggesting homogeneity among adiposity levels and ethnic groups may be extended to all of the family studies in Table 5, except for the Mexican American (12) and Pima Indian (17) samples. We note that these two studies are unique, based on Hispanic and Native American groups that are characterized by greater degrees of overweight, obesity, and diabetes than much of the remaining North American population (28). As discussed previously, the effects of impaired glucose tolerance or insulin resistance on leptin secretion are not clear in humans (8,10), and this (i.e., genetic heterogeneity) may have contributed to the differences in the heritabilities.

Genetic heterogeneity across ethnic groups can arise if certain allele forms are more prevalent in some but not all

Table 3. Heterogeneity of goodness of fit tests (*p* values)

Subsamples and test	*df*	Age	Age-SF6
Among 4 Clinics in HERITAGE			
Spouse heterogeneous	3	0.4219	0.7244
P-O heterogeneous	12	0.2900	0.2623
Sibling heterogeneous	9	0.7218	0.1195
Among 4 Subsamples			
Spouse heterogeneous	3	0.5499	0.0157
P-O heterogeneous	12	0.1982	0.0878
Sibling heterogeneous	9	0.1056	0.1969
Random vs. obese (QFS)			
Spouse heterogeneous	1	0.2435	0.0057
P-O heterogeneous	4	0.1348	0.7670
Sibling heterogeneous	3	0.1161	0.2669
Blacks vs. whites (HERITAGE)			
Spouse heterogeneous	1	0.3055	0.5598
P-O heterogeneous	4	0.1065	0.3570
Sibling heterogeneous	3	0.3780	0.1068

Eight parameters (FM, FS, FD, MS, MD, SS, DD, SD) were measured for each sample: FM, father-mother; FS, father-son; FD, father-daughter; MS, mother-son; MD, mother-daughter; SS, son-son; DD, daughter-daughter; SD, son-daughter. *df*, degrees of freedom; P-O, parent-offspring; SF6, skinfold sum.

groups. This would apply not only to the known leptin locus, but also to quantitative trait loci (QTLs) for other traits that can modify leptin levels such as insulin or obesity. Although there was no suggestion of genetic heterogeneity across the ethnic groups in the current study, we cannot rule out this possibility for others such as the Mexican American (12) and Pima Indian (17) groups. More evidence for genetic heterogeneity can be inferred from methods such as genome scan linkage analysis. For example, if some QTLs are consistently observed across different samples, then their effects may be homogeneous across groups. On the other hand, regions that appear only in certain groups can reflect genetic heterogeneity or could simply be a result of type I errors (i.e., false positives).

The possibility of genetic homogeneity for some QTLs, as well as heterogeneity for others, is observed in several recent genome scan studies (12,17,19,29–33). First, it is interesting to note that none of these scans provided strong linkage evidence for leptin at or near the leptin locus (7q31) or the leptin receptor (1p31), although there is linkage evidence in these regions for some adiposity measures (2). Second, one region appears to reflect homogeneity because it has been replicated across several samples: 2p21 is linked in Mexican American (12), black (29), and French samples (30). Thus, the 2p region may contain a QTL that is homogeneous across many different groups. There is some evidence that the POMC locus, involved in the neuroendocrine system, may be responsible for this signal in Mexican Americans (31). Third, other linkage regions reported in specific groups have not replicated across samples. For example, the 5p11 and 11p15 regions were linked in French samples (30,32), 6p21 and 16q21 in Pima Indians (17), 8q11 in Mexican Americans (12), 3q and 17p in the Take Off Pounds Sensibly (TOPS) obese white sample (19), and 19p in the HERITAGE white families (33). The fact that some of these regions (6p21, 8q11, 11p15, and 19p) also show linkage for various adiposity measures (2) and some (6p21, 11p15, and 16q22) for type 1 diabetes (34,35) lends support to the hypothesis that these QTLs reflect a real signal (rather than a type I error) and may contribute to genetic heterogeneity for leptin levels directly or indirectly via obesity or insulin levels. These hypotheses warrant further investigation.

An additional question regarding the adiposity-leptin relationship is the effect of adiposity adjustment on the resulting leptin heritabilities. In general, if traits A and B are influenced by genes, and if some of those genes affect both traits (i.e., pleiotropy), then removing the affect of A from B could alter the heritability estimate for B. The direction of the effect would depend on how genes for A and B interact to influence the trait in question. In this study, there was a slight drop in the magnitude of the heritability after adiposity adjustment, but this difference was not significant based on a SE comparison. Three other studies reported analyses before and after adiposity adjustment. In all cases there was a reduction in the familial effect after adjustment, from 73% to 55%, in a study by Narkiewicz et al. (13), from 21% to 0 in a study by Walder et al. (17), and from 65% to ~40% in a study by Jenkins et al. (18). However, in one of these studies (18), the effects of age were confounded with the adiposity adjustment, and failure to adjust for age in itself could have inflated the heritability. In the remaining studies, this pattern of a drop in heritability after removing the variance due to adiposity levels is consistent with additive pleiotropy. This pleiotropic hypothesis was specifically investigated in a bivariate analysis (15) of twin data. Results suggested that the additive genetic components underlying leptin and BMI were highly but not completely correlated (r_g ~0.70). That is, apparently some of the same genes influence both traits, whereas other genes affect only one trait or the other. We further note that this pleiotropic effect between adiposity and leptin levels may be related to initial levels of adiposity. For example, the studies showing significant reductions in heritabilities after adiposity adjustment were based on samples with more extreme mean BMIs (36 vs. 21 kg/m^2) compared with the present study (32–35),

Table 4. Hypothesis testing and most parsimonious models

	Hypothesis testing						
	Age		Age-SF6		Most parsimonious models		
Groups/model	*p* value	AIC	*p* value	AIC	Parameters	Age	Age-SF6
1. General*		16.00	20.00				
2. No sex difference in offspring	0.6644	10.39	0.4225	15.88	Spouse		
3. No sex difference in offspring/parents	0.7810	8.47	0.3111	15.95	FM_1 (QFS random)	0.16 ± 0.06	0.42 ± 0.09
4. No sex or generational differences	0.5425	9.01	0.0364	21.45	FM_2 (QFS obese)	[0.16]	[0]
					$FM_3 = FM_4$		
5. No spouse correlation	0.0203	19.39	0.0012	29.80	(HERITAGE)	[0.16]	0.12 ± 0.11
5a. $FM_1 = 0$			0.0002	31.46	Parent-Offspring		
5b. $FM_2 = 0$			0.2617	19.26	FS = FD = MS = MD	0.20 ± 0.03	0.22 ± 0.03
5c. $FM_3 = FM_4 = 0$			0.0098	24.68	Sibling		
6. No parent-offspring correlation	<0.0001	50.76	<0.0001	59.15	SS = DD = SD	0.14 ± 0.04	0.09 ± 0.04
7. No sibling correlation	0.0009	26.50	0.0839	20.65	Maximal heritability†		
8. Single correlation	<0.0001	33.75	0.0007	30.95	h^2 (QFS random)	32% ± 0.07	27% ± 0.07
9. No familial correlations	<0.0001	62.66	<0.0001	73.75	h^2 (QFS obese)	[32%]	31% ± 0.07
10. Most parsimonious:							
3 + 5b + 7			0.0702	19.08	h^2 (HERITAGE)	[32%]	30% ± 0.07
3 + 5b			0.3246	14.96	h^2 (weighted average)		29% ± 0.07
3	0.7810	8.47					

AIC, Information Criterion from Akaike; QFS, Québec Family Study.

* For age-adjusted leptin, the general model included eight parameters (FM, FS, FD, MS, MD, SS, DD, SD); for age- and SF6-adjusted leptin, the general model incorporated 10 parameters including 2 additional spouse correlations (i.e., FM_1, FM_2, $FM_3 = FM_4$, FS, FD, MS, MD, SS, DD, SD).

† Maximal heritability (h^2) was computed as: $[(r_{sib} + r_{parent\text{-}offspring})\ (1 + r_{spouse})]/[(1 + r_{spouse}) + (2)\ (r_{spouse})\ (r_{parent\text{-}offspring})]$. This estimate is adjusted for the degree of spouse resemblance (if significant) and reduces to twice the average parent-offspring and sibling correlation if spouse resemblance is not significant (27). Estimates ± SE.

suggesting that any genetic component underlying the covariation between leptin and BMI may be more pronounced at extreme adiposity levels.

At the individual or phenotypic level, regression analyses support an important role for adiposity in leptin values, accounting for up to 70% of the variance. We noted that exploratory stepwise regressions (not reported) were performed with other adiposity measures (underwater weighing measures of fat mass and fat-free mass, computed tomography scans of visceral and subcutaneous abdominal fat, BMI, and waist-to-hip ratio). Results suggested that total subcutaneous fat (SF6) accounted for the most variance, and no other adiposity variable entered the stepwise model when SF6 was included. This supports some other studies (36) suggesting that the subcutaneous depot is a better determinant of leptin concentrations. Of the remaining variance in leptin not explained by age and adiposity, ~30% was due to heritable factors. When expressed as a percentage of the total variance, the heritability is ~10% suggesting that ~20% of the total variance is still unaccounted for.

Finally, we note that about one-half of the studies in Table 5 performed a log transform on leptin levels before genetic analyses. In the HERITAGE study and QFS, leptin was significantly skewed and leptokurtotic, and a log transform produced a normally distributed trait. The magnitudes of the heritabilities in Table 5 do not seem to be related to whether a log transform was conducted. In fact, the single study that reported results before and after such an adjust-

Table 5. Studies reporting heritability for leptin levels

Reference no.	Number of pedigrees (individuals) and sample description	Method	Transform/ adjustment	Mean BMI	Heritability (±SE)
Twins					
Narkiewicz (13)	19 MZ and 14 DZ Polish twin pairs	VC	Log/none	21	73%
			Log/BMI		55%
Jenkins (18)	127 MZ and 400 DZ British twin pairs*	VC	/none	17–44	60%
			Log/none		65%
			Log/age, sex, FM		36%
			Log/age, sex, BMI		42%
Kaprio (15)	58 MZ and 74 DZ Finnish twin pairs, non-diabetic†	VC	/Age, sex	27	45% men, 34% women
Families					
Comuzzie (12)	10 (458) Mexican American pedigrees‡	VC	Not reported	>50% overweight	63% ± 0.10
Luke (16)	(1556) U.S. black families	VC	/Age, sex	29	43% ± 0.06
Hsueh (14)	45 (953) Amish pedigrees selected for diabetes	VC	Log/age, sex	27	42% ± 0.07
Rotimi (11)	118 (361) U.S. black families	FC	/Age, sex	30	39% ± 0.11
Luke (16)	(469) Nigerian black families	VC	/Age, sex	21	38% ± 0.14
Kissebah (19)	507 (2209) white families selected for obesity (TOPS)§	VC	/Age, sex, smoke, & menstrual	32	32% ± 0.06
Current study	432 (1432) N. Amer. black, white, random and obese families	FC	Log/age, sex	25–32	32% ± 0.07
			Log/age, sex, SF6		29% ± 0.07
Luke (16)	(457) Jamaican black families	VC	/Age, sex	26	25% ± 0.12
Walder (17)	239 (770) Pima Indians, pedigrees, non-diabetic	VC	Log/age, sex	36	21% ± 0.08
			Log/age, sex, BMI		0%

BMI, body mass index; SF6, skinfold sum; FM, fat mass; VC, variance components; FC, familial correlations.

* Range of BMI given as mean not reported.

† Age not a significant predictor; analyzed separately by sex. Genetic correlation between BMI and leptin was 0.79 in women and 0.68 in men.

‡ Overweight at age 40 defined as BMI >29.5 for men and >31.0 for women.

§ Take Off Pounds Sensibly (TOPS), families with at least two obese siblings (BMI ≥30) and an additional lean (BMI ≤27) sibling or parent.

ment (18) provided similar estimates (60% vs. 65% in twins). Although the heritability methods are generally robust against deviations from normality, simulations by Blangero et al. (37) suggest that leptokurtic distributions can yield excessive type I error rates in linkage test statistics. Because follow-up studies involving the HERITAGE study and QFS will involve linkage analysis to isolate the sources of the signals reported here, we corrected for the leptokurtosis.

In summary, our estimate of 32% heritability for leptin is based on one of the larger samples reported to date. This estimate did not change across adiposity levels or black vs. white ethnic groups, nor was it different from the weighted

average across other family studies of 35% to 36%. The previous reports that diverged from this estimate included some twin studies and two family studies. Methodological differences most likely are the cause for the different twin estimates. However, statistical or methodological differences did not seem to contribute to the differences observed for the two family studies. Rather, these two studies were based on unique ethnic groups (Hispanic and Native American) in which there is an over-representation of obesity and diabetes, both of which can influence leptin levels in humans. Thus, genetic heterogeneity cannot be ruled out for these ethnic groups. Additional work is needed to test if our conclusions apply to other ethnic groups or to extreme levels of adiposity not indexed here and how interactions between diabetes and adiposity affect leptin variation.

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