Evidence of Pleiotropic Loci for Fasting Insulin, Total Fat Mass, and Abdominal Visceral Fat in a Sedentary Population: The HERITAGE Family Study

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Abstract

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Objective: To examine whether there is a major gene effect on fasting insulin and pleiotropic loci for fasting insulin, total fat mass (FM), and abdominal visceral fat (AVF).

Research Methods and Procedures: A major gene hypothesis for fasting plasma insulin levels was assessed using segregation analyses of data on 495 members in 98 normolipidemic sedentary families of white descent who participated in the HERITAGE Family Study.

Results: Segregation analyses were performed on insulin adjusted for age, on insulin adjusted for age and FM, and on insulin adjusted for age and AVF. Before adjustment for AVF and FM, a major gene effect on fasting insulin levels was indicated. The putative locus accounted for 54% of the variance under a recessive inheritance pattern, affecting 11% of the sample (i.e., allele frequency = 0.33). However, after adjusting for the effects of AVF or FM, neither a major effect alone nor a multifactorial component alone could be rejected, and support for a major gene was equivocal, i.e., neither the hypothesis of Mendelian τ values or that of the equal τs were rejected and the equal τ model fit the data better than the Mendelian τ model. This pattern (i.e., major gene evidence for insulin before but not after adjustment for AVF or FM) suggests that there is a putative locus with pleiotropic effects on both insulin and FM and another pleiotropic locus for both insulin and AVF.

Discussion: Although these data do not directly support an additional major gene for insulin independent of AVF and FM, such support cannot be ruled out because there is still a significant major effect on FM- or AVF-adjusted insulin (albeit the Mendelian nature of this effect is ambiguous).

Key words: segregation analysis, genetic epidemiology, insulin resistance, obesity, diabetes

Introduction

Type 2 diabetes is one of the most common chronic diseases in our society (1,2). Diabetes is characterized by abnormal elevations in plasma glucose levels and is caused either by a deficiency of insulin, by resistance to the action of insulin, or by decreased effectiveness in glucose uptake and suppression of hepatic glucose output (3,4). Insulin resistance is among one of the most important predictors for type 2 diabetes and coronary heart disease (5–10) and is believed to precede the development of the clinical manifestations of type 2 diabetes and coronary heart disease by many years. There are several indices that have been used to represent insulin resistance (11–15). Although impaired insulin sensitivity measured from glucose clamping is the

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standard method used for assessing insulin resistance (12), fasting insulin levels are still widely used in epidemiological studies, and the two methods are highly correlated in subjects with normal glucose tolerance (16).

Among many other factors, genetic components have been reported to play important roles in variation of insulin resistance (17–21). Heritability estimates for fasting insulin and insulin resistance ranged from moderate to high. For example, in a study of a European population, an autosomal recessive gene was indicated for insulin response to glucose (22), whereas in non-diabetic Pima Indians, commingling analysis suggested that a mixture of three distributions best fit the data, consistent with a pattern of a co-dominant major gene (23). Using segregation analysis, a major gene for fasting insulin was found in non-Hispanic white families of Utah areas that were ascertained through type 2 diabetic probands (24); however, the major gene effect was only found after adjustment for both age and body mass index (BMI). In a Mexican American population, a major gene model for fasting insulin was not supported, although there was clear evidence of a major gene effect for 2-hour insulin levels measured after an oral glucose tolerance test (25). It appears that the evidence for a major gene effect depends on the populations studied and the adjustments for other covariates such as adiposity indices. Among the adiposity phenotypes, total body fat mass (FM) and abdominal visceral fat (AVF) are considered important indicators of metabolic disturbances leading to insulin resistance (26–28).

In the present investigation, univariate segregation analysis of fasting insulin was applied to 495 subjects from 98 normolipidemic sedentary white families who participated in the HERITAGE Family Study. A moderate heritability of fasting insulin was found using a familial correlation model in our previous investigation of this same sample (29). Univariate segregation analyses of a trait both before and after adjustment for covariate effects can also be used to evaluate whether there is a pleiotropic effect on the trait and the covariate, although indirectly. Here, the effect of a major gene on fasting insulin levels was investigated both before and after removing the effects of FM or AVF. If there is a major gene for plasma insulin that also affects AVF or FM, then the segregation patterns for insulin levels should differ depending on whether or not it is adjusted for AVF or FM. A difference in pattern could provide an indication as to how these traits interact with each other from a genetic perspective.

Research Methods and Procedures

Study Subjects

The HERITAGE Family Study is a multicenter exercise study involving families. The main objective of the study is to assess the role of genetic factors in the cardiovascular, metabolic, and hormonal responses to aerobic exercise training in sedentary families. The HERITAGE sampling procedure and the inclusion and exclusion criteria have been described in detail elsewhere (30).

In brief, several criteria were used to screen subjects and families for participation. First, offspring were required to be between the ages of 17 and 40 years, and parents were required to be 65 years old or less, in order to minimize possible complications associated with subjects undergoing growth (low end) and aging (high end). Second, families were required to be sedentary, defined at baseline as no regular strenuous physical activity over the previous 6 months (30). Third, individuals with a BMI greater than 40 kg/m^2 were usually excluded unless they were deemed fit (by a physician) to exercise on a cycle ergometer without difficulties. Fourth, individuals with blood pressures greater than 159 mm Hg systolic and/or 99 mm Hg diastolic were also excluded. Fifth, individuals with any life-threatening condition or disease, or a condition that could be aggravated by cycle exercise, were excluded (e.g., a malignancy, uncontrolled endocrine, and metabolic disorders, including diabetes, definite or possible coronary heart disease, and chronic or recurrent respiratory problems). Finally, individuals taking lipid-lowering or antihypertensive drugs were excluded.

In all, 98 nuclear families of white descent, each with both biological parents and at least two biological children (most often three or more), completed the protocol. Data from African American families were also collected but not reported in the present investigation.

Measurements

All participants underwent a series of tests both before and after completing a 20-week standardized exercise training program (see Reference 30 for details). Results from the baseline (pre-exercise training) tests are reported in the present study.

Fasting Plasma Insulin. Blood samples were collected under EDTA and the tubes were centrifuged at 1000*g* at a temperature of 4 °C for 10 minutes. Plasma was kept frozen at −20 °C until the time of assay. Plasma insulin levels were measured by radioimmunoassay after polyethylene glycol separation as described by Desbuquois and Aurbach (31). Polyclonal antibodies, which cross-react more than 90% with proinsulin and presumably its conversion intermediates, were used (32). Therefore, in this study as in others (32,33), insulin refers to immunoreactive insulin defined as the sum of insulin, proinsulin, and split-proinsulin. In the present cohort with normal fasting glucose levels and no history of diabetes, it is estimated that about 10% of the immunoreactive insulin is in the form of proinsulin and its conversion intermediates (33). Insulin levels were treated as missing for three individuals with insulin antibodies, four individuals with extremely low glucose disappearance

rate, and one individual with both conditions. All the assays were performed at a central laboratory in Québec. The intra- and interassay coefficients of variation were 7.7% and 10.3%, respectively.

Abdominal Visceral Fat. AVF levels were measured by computerized tomography scanning (34). Subjects were examined in a supine position with their arms stretched above the head. The abdominal scan was obtained between the fourth and fifth lumbar vertebrae. The attenuation interval used in the quantification of the areas of adipose tissue ranged from −190 to −30 Hounsfield units. The AVF area was defined by drawing a line within the muscle wall surrounding the abdominal cavity.

Total Body Fat Mass. Underwater weighing was performed to determine total body FM (35). A correction was made for residual lung volume by oxygen dilution methods (36). At the Laval University Clinical Center, residual lung volume was assessed by the helium dilution technique (37).

Data Adjustments

Insulin levels were analyzed under two different adjustment schemes. First, insulin levels were adjusted for a polynomial in age (age, age^2, and age^3) using stepwise multiple regression. Second, insulin levels were adjusted for the linear effects of FM in addition to the polynomial effects of age. Third, insulin levels were adjusted for the linear effects of AVF in addition to the polynomial effects of age. All the data adjustments were carried out separately in the four sex-by-generation groups (fathers, mothers, sons, and daughters) because significant group differences in the means were noted. The significance level for retaining terms in the stepwise regression analysis was 5%.

Segregation Analysis

The computer program POINTER (38) was utilized to carry out the segregation analyses. A more detailed explanation of the segregation analysis method used here is given in the Appendix. The overall mean (u) and variance (V), heritable multifactorial effects in offspring (H) and parents (HZ), and parameters of the major gene component (single bi-allelic locus) are estimated. The major effect is represented by three parameters: q, which determines the relative proportion (q^2) of the component distribution with the highest (homozygote) mean; t, which is the displacement between the two extreme (homozygote) component means; and d, which is the relative position of the middle (heterozygote) component mean (i.e., $d = 0$ is recessive and $d = 1$ is dominant). Three transmission probabilities (τ_1, τ_2, and τ_3) are estimated to test whether the major effect is transmitted in families according to Mendelian expectations (see Appendix).

Results

Descriptive Results

Table 1 presents the means and SDs of the unadjusted levels of fasting insulin, AVF, FM, and BMI in the four sex-by-generation groups. Based on a comparison of means and SEs, males have higher fasting insulin, ($p < 0.001$), AVF, ($p < 0.001$), and BMI ($p < 0.001$) than females, while there is no significant sex difference in FM ($p = 0.15$). In addition, there is a phenotypic correlation between fasting insulin and FM levels ($r = 0.47$, $p < 0.01$ for males; $r = 0.35$, $p < 0.01$ for females). The correlation between fasting insulin and AVF is also significant ($r = 0.43$, $p < 0.01$ for males; $r = 0.31$, $p < 0.01$ for females), as is the correlation between FM and AVF ($r = 0.69$, $p < 0.01$).

Segregation Analysis

Segregation analysis results are given in Table 2 for insulin levels adjusted for age, in Table 3 for insulin levels adjusted for age and FM, and in Table 4 for insulin levels adjusted for age and AVF. For age-adjusted insulin, the test for no multifactorial effect (model 2 vs. model 1) was not rejected ($p < 0.24$), whereas the test for no major effect (model 3 vs. model 1; $p < 0.01$) was rejected. This suggested that there is a major effect for fasting insulin levels.

Under the general Mendelian model with no multifactorial component, a recessive mode of inheritance for the major effect could not be rejected ($d = 0$: model 5 vs. model 2; $p = 0.73$), whereas neither additive ($d = ½$: model 6 vs. model 2; $p < 0.01$) nor dominant modes ($d = 1$: model 7 vs. model 2; $p < 0.01$) fit the data. Finally, tests on the transmission probabilities suggest that the hypothesis of Mendelian τ values (model 8 vs. model 9; $p = 0.61$) was not rejected, whereas that of equal τs (model 10 vs. model 9; $p = 0.02$) was rejected. These results satisfy all the requirements needed to infer a major gene: 1) rejection of the hypothesis of no major effect; 2) non-rejection of the hypothesis of Mendelian τs; and 3) rejection of the hypothesis of no transmission of the major effect. Under the most parsimonious model, the major gene effect accounts for 54% of the variance of fasting insulin levels, affecting 11% of the population.

Results for age- and FM-adjusted insulin levels are given in Table 3. Both hypotheses of a major effect only (model 2 vs. model 1; $p < 0.01$) and a multifactorial effect only (model 3 vs. model 1; $p < 0.01$) were rejected, suggesting that both components are significant. The test of $Z = 1$ (model 5 vs. model 1; $p = 0.72$) was not rejected, indicating there was no generation difference in the multifactorial effect. Further tests on the nature of the major effect show that a recessive mode of transmission (model 6 vs. model 5; $p = 1.00$) was not rejected, whereas additive (model 7 vs. model 5; $p < 0.01$) and dominant (model 8 vs. model 5; $p < 0.01$) modes of transmission were rejected. The hypothesis of Mendelian τs under the parsimonious Mendelian model

Table 1. Characterics of age, plasma insulin, AVF, and FM by sex and generation groups

Variable	Group	*N*	Mean	SD
Fasting insulin (pM)	Fathers	90	78.28	59.04
	Mothers	90	61.81	29.25
	Sons	151	67.23	40.77
	Daughters	166	58.73	26.36
AVF (cm^2)	Fathers	95	156.68	61.17
	Mothers	92	120.18	59.35
	Sons	155	77.11	43.52
	Daughters	166	52.20	28.77
FM (kg)	Fathers	89	24.67	9.08
	Mothers	85	26.98	10.38
	Sons	146	16.90	11.10
	Daughters	167	18.01	9.77
BMI (kg/m^2)	Fathers	95	28.28	4.48
	Mothers	93	27.65	4.98
	Sons	157	25.65	4.92
	Daughters	167	23.68	4.44

(model 9: no generation difference in the multifactorial component and a recessive mode of inheritance for the major effect component) was rejected (model 9 vs. model 10; $p = 0.04$), whereas the equal τs model was not rejected (model 11 vs. model 10; $p = 0.65$). Thus, for age- and FM-adjusted insulin levels, both the multifactorial and major components are significant, although there is no transmission of the latter component and therefore a major gene effect cannot be inferred.

Results of segregation analysis for age- and AVF-adjusted insulin levels are similar to those for age- and FM-adjusted insulin levels (Table 4). Both a major effect only (model 2 vs. model 1; $p < 0.01$) and a multifactorial effect only (model 3 vs. model 1; $p < 0.01$) were rejected. There is no generation difference in the multifactorial effect (model 5 vs. model 1, $p = 0.45$) and a recessive mode of inheritance for the major effect component (model 6 vs. model 5, $p = 1.00$) best fit the data. Also, the hypothesis of Mendelian τs under the parsimonious Mendelian model was rejected (model 9 vs. model 10; $p = 0.02$), whereas the equal τs model was not rejected (model 11 vs. model 10; $p = 0.30$). Thus, a major gene for age- and AVF-adjusted insulin levels is not supported.

The segregation analysis results clearly indicate a major gene effect for age-adjusted insulin levels, whereas there is no evidence of major gene effect for age- and FM-adjusted insulin levels or age- and AVF-adjusted insulin levels. Together, these findings suggest that there is evidence of a pleiotropic locus for insulin and FM and another pleiotropic locus for insulin and AVF. The non-Mendelian major effect found for age- and FM-adjusted insulin and age- and AVF-adjusted insulin levels could be environmental, but a major gene that interacts with other unmeasured factors (genetic or environmental) cannot be ruled out.

Discussion

The present segregation analyses were performed to assess whether there is a major gene effect on insulin levels and whether oligogenic-pleiotropic mechanisms are involved in the relationship between levels of fasting insulin, FM, and AVF in an adult normolipidemic sedentary population by comparing the results for insulin levels before and

Table 2. Segregation analysis of insulin levels adjusted for age

Model	*d*	*t*	*q*	*H*	*Z*	−2lnL+c	Model comparison	χ^2		*p*
1. General model	0.00	3.12	0.29	0.12	1.16	−1.12				
2. Major effect only	0.02	2.95	0.33	[0]	[0]	1.72	vs. model 1	2.84	2	0.24
3. Multifactorial effect only	[0]	[0]	[0]	0.13	0.45	66.46	vs. model 1	67.58	3	<0.01
4. No familial effect	[0]	[0]	[0]	[0]	[0]	77.26	vs. model 1	78.38	5	<0.01
5. Recessive	[0]	2.91	0.33	[0]	[0]	1.84	vs. model 2	0.12	1	0.73
6. Additive	½	4.12	0.06	[0]	[0]	41.72	vs. model 2	40.00	1	<0.01
7. Dominant	[1]	2.39	0.08	[0]	[0]	32.39	vs. model 2	30.67	1	<0.01
8. Parsimonious Mendelian*	[0]	2.91	0.33	[0]	[0]	1.84	vs. model 9	1.84	3	0.61
9. Free τs†	[0]	2.92	0.22	[0]	[0]	0.00				
10. Equal τs‡	[0]	2.99	0.30	[0]	[0]	9.43	vs. model 9	9.43	3	0.02

* $V = 1.52$, $u = 0.15$, and 54% of the variance due to the major locus under parsimonious Mendelian model.
† $\tau_1 = 0.82$, $\tau_2 = 0.59$, $\tau_3 = 0.13$.
‡ $\tau_1 = \tau_2 = \tau_3 = 0.71$.

Table 3. Segregation analysis of insulin levels adjusted for age and total body fat

Model	*d*	*t*	*q*	H	*Z*	−2lnL+c	Model comparison	χ^2	*df*	*p*
1. General Mendelian	0.00	3.32	0.26	0.25	0.88	8.23				
2. Major effect only	0.17	3.21	0.32	[0]	[0]	37.00	vs. model 1	28.77	2	<0.01
3. Multifactorial effect only	[0]	[0]	[0]	0.30	0.65	76.54	vs. model 1	68.31	3	<0.01
4. No familial effect	[0]	[0]	[0]	[0]	[0]	93.67	vs. model 1	85.44	5	<0.01
5. $Z = 1$	0.00	3.33	0.26	0.24	[1]	8.36	vs. model 1	0.13	1	0.72
6. Recessive	[0]	3.33	0.26	0.24	[1]	8.36	vs. model 5	0.00	1	1.00
7. Additive	½	0.93	0.02	0.24	[1]	76.76	vs. model 5	68.40	1	<0.01
8. Dominant	[1]	1.79	0.09	0.08	[1]	58.03	vs. model 5	56.65	1	<0.01
9. Parsimonious Mendelian*	[0]	3.33	0.26	0.24	[1]	8.36	vs. model 10	8.36	3	0.04
10. Free τs†	[0]	3.20	0.19	0.31	[1]	0.00				
11. Equal τs‡	[0]	3.17	0.23	0.26	[1]	1.66	vs. model 10	1.66	3	0.65

* $V = 1.42$, $u = 0.12$, under parsimonious Mendelian model.
† $\tau_1 = 0.89$, $\tau_2 = 0.52$, $\tau_3 = 0.68$.
‡ $\tau_1 = \tau_2 = \tau_3 = 0.77$.

after adjustment for FM or AVF. Before adjustment for FM or AVF, there was a clear evidence of a major gene effect for fasting insulin, whereas multifactorial familial effects were not significant. The major gene effect accounted for 54% of the variance and affected 11% of the sample. However, it is interesting to note that, after adjusting for FM or AVF, the evidence of a putative major locus for the insulin levels was equivocal because the Mendelian transmission could not be inferred. This pattern suggests that there is a major gene with pleiotropic effects on insulin levels and FM and possibly another one for insulin and AVF. In addition, there is evidence of a non-Mendelian major effect that explains the residual familial effects on insulin levels. The non-Mendelian major effect could pos-

Table 4. Segregation analysis of insulin levels adjusted for age and abdominal visceral fat

Model	*d*	*t*	*q*	*H*	*Z*	−2lnL+c	Model comparison	χ^2	*df*	*p*
1. General Mendelian	0.00	3.33	0.28	0.23	0.65	9.19				
2. Major effect only	0.18	3.73	0.27	[0]	[0]	21.03	vs. model 1	11.84	2	<0.01
3. Multifactorial effect only	[0]	[0]	[0]	0.22	1.76	72.88	vs. model 1	63.69	3	<0.01
4. No familial effect	[0]	[0]	[0]	[0]	[0]	92.32	vs. model 1	83.13	5	<0.01
5. $Z = 1$	0.00	3.37	0.27	0.20	[1]	9.75	vs. model 1	0.56	1	0.45
6. Recessive	[0]	3.37	0.27	0.20	[1]	9.75	vs. model 5	0.00	1	1.00
7. Additive	½	0.81	0.50	0.22	[1]	73.72	vs. model 5	63.97	1	<0.01
8. Dominant	[1]	2.38	0.07	0.10	[1]	52.10	vs. model 5	42.35	1	<0.01
9. Parsimonious Mendelian*	[0]	3.37	0.27	0.20	[1]	9.75	vs. model 10	9.75	3	0.02
10. Free τs†	[0]	3.30	0.15	0.27	[1]	0.00				
11. Equal τs‡	[0]	3.30	0.23	0.21	[1]	3.75	vs. model 10	3.75	3	0.30

* $V = 1.56$, $u = 0.16$, under parsimonious Mendelian model.
† $\tau_1 = 0.79$, $\tau_2 = 0.79$, $\tau_3 = 0.29$.
‡ $\tau_{\tau_1} = \tau_2 = \tau_3 = 0.77$.

sibly be explained by multifactorial factors. However, we cannot rule out the possibility of a second major gene that interacts with other unknown covariates.

There are several reports on the segregation analyses of fasting insulin levels and insulin resistance. A major gene effect for insulin response to glucose was noted in a European population. A major gene for fasting insulin was also found in Pima Indians and non-Hispanic whites (22–24) but not in Mexican Americans (25). Moreover, a co-dominant pattern was observed in the Pima Indians, whereas a recessive pattern was noted in the white populations. Different study designs and different study populations may explain the different results. First, the genetic basis for insulin resistance could vary in different ethnic groups. Additionally, different subgroups in the same ethnic groups may also differ in their genetic background. For example, clinical and epidemiological studies show that non-diabetic Pima Indians and Mexican Americans have higher fasting insulin levels than do non-Hispanic whites (39). Insulin resistance also occurs more often in blacks than in whites (40,41). Furthermore, obese and sedentary populations have higher fasting insulin levels than non-obese and physically active populations (42). In the present investigation of non-Hispanic whites, 11% were homozygous recessive for hyperinsulinemia. This frequency is slightly higher than that for non-Hispanic whites from the Utah study (6.25%) but lower than that for Pima Indians. Our higher frequency in comparison with the Utah Study could be partly due to our ascertainment from a sedentary population, especially if inactivity is due in part to genetic influence (43) and inactivity is related to insulin resistance or type 2 diabetes (44). The difference between whites and Pima Indians could be due to the fact that Pima Indians tend to be more obese and have a high prevalence of type 2 diabetes.

Hyperinsulinemia and obesity are strongly associated (42). The Utah Study also investigated the relationship between insulin levels and obesity (24). A major gene affecting insulin was detected only after the variance in insulin levels attributable to BMI was removed. This pattern is consistent with a potential oligogenic model for insulin with additional common familial factors affecting both insulin and BMI. That is, before adjusting insulin for BMI, the major gene evidence was equivocal, although there were significant familial effects. After adjustment for BMI, a major gene effect was apparent (i.e., oligogenic). The different results before and after adjustment for BMI also indirectly suggest the evidence for pleiotropy. The source of the common familial component could be multifactorial, or it could be due to another major locus that is influenced by other unmeasured covariates. Our results in the HERITAGE Family Study suggest that major loci with pleiotropic effects are partly responsible for the common familial effects for fasting insulin, FM, and AVF. In addition, the residual familial effect on insulin levels (after accounting for that locus, if any) may be a function of polygenic and/or environmental factors. However, the possibility that this residual familial effect is due to a second locus (oligogenic) that interacts with other unknown covariates cannot be ruled out.

The differences in findings between the Utah Study (24) and the HERITAGE Family Study may be simply a function of different samples in which there are different allele frequencies and/or genotypes. In addition, the choice of obesity phenotypes may be relevant. It is known that the BMI is a generalized measure of body composition and incorporates bone and muscle mass as well as FM. FM and AVF, on the other hand, are strictly measures of total FM and FM in the abdominal visceral area. In our previous investigation (45) and the Québec Family Study (46), a major gene effect for AVF was indicated. A major gene with pleiotropic effect on AVF and FM was also indicated in our previous study (45).

Figure 1 illustrates a hypothetical model accounting for the oligogenic-pleiotropic genetic evidence for fasting insulin, FM, and AVF found in the present study and our previous studies. First, there is a major gene with pleiotropic effect (G_{p1}) for levels of fasting insulin and FM and a second pleiotropic locus (G_{p2}) for fasting insulin and AVF. Second, there is a possible additional major gene (G_3) for fasting insulin independent of FM and AVF. Third, given the report by Rice et al. (45) and Bouchard et al. (46), the possibility is that G_{p1} and G_{p2} are the same gene or at least highly correlated. Fourth, the pleiotropic and independent environmental effects on levels of fasting insulin, FM, and AVF are not illustrated because they were not tested in the present study. Fifth, at the phenotypic level, it is likely that accumulation of FM is directly or indirectly (for example, via increased levels of AVF) involved in the regulation of fasting insulin levels. The pleiotropic genetic effects for fasting insulin and FM may be responsible for the overall fat accumulation and increased insulin levels, whereas the pleiotropic loci for fasting insulin and AVF may be related mainly to fat distribution and tissue-specific insulin resistance. In support of our findings of pleiotropic genetic effects for insulin and FM or AVF, one locus on human chromosome 11, namely the uncoupling protein-2, has recently been reported to be linked to hyperinsulinemia and obesity (47). However, in two other recent studies on Pima Indians, evidence of linkage for fasting insulin was found at 3q21-24 and 4p15-q12 (48), whereas evidence of linkage for percentage body fat was found at 11q21-q22 and 18q21 (49).

Confirmation of these linkage results in other studies is needed. In addition, whether these linkage results stand after adjusting insulin levels for FM and/or AVF will also add to our understanding of the function of these genes. Molecular studies to address these issues in the HERITAGE Family Study are currently underway.

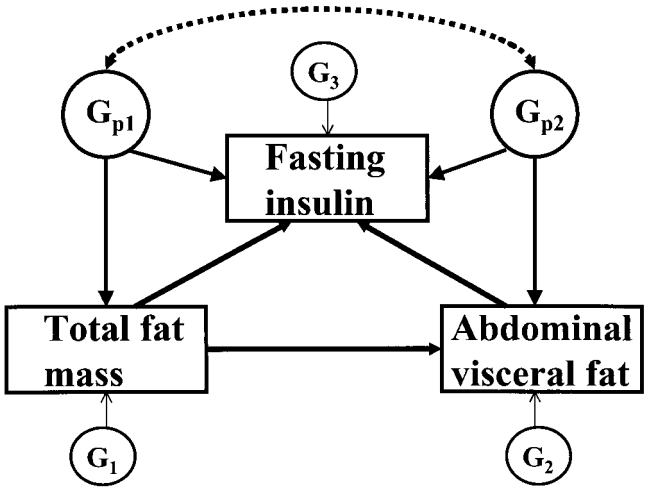

Figure 1. Oligogenic-pleiotropic genetic effects on levels of fasting insulin, total body FM, and AVF. G_{p1} represents a pleiotropic locus for fasting insulin and FM; $G_{p2,}$ a pleiotropic locus for fasting insulin and AVF; the correlation between G_{p1} and G_{p2} may be 1.0, indicating that these may be the "same" gene; G_1, a major gene for FM; G_2, a major gene for AVF; G_3, a possible major gene for fasting insulin.

In conclusion, there is evidence for existence of a major gene with pleiotropic effects on levels of fasting insulin and FM and a possible second pleiotropic locus for fasting insulin and AVF. These two pleiotropic loci could be the same gene or highly correlated. Although these data do not clearly support a gene effect on insulin independent of AVF and FM, presence of such a gene cannot be ruled out because there is still a significant major effect on FM- or AVF-adjusted insulin (albeit the Mendelian nature of this effect is ambiguous).

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Appendix: Segregation Analysis

Segregation analysis was carried out using the unified mixed model (50) as implemented in the computer program POINTER (38). The general model assumes that a phenotype is composed of the independent and additive contributions from a major gene effect, a heritable multifactorial background, and a unique environmental component (residual). The major effect is assumed to result from the segregation at a single locus having two alleles (i.e., *A* and *a*). The *A* allele is defined as decreasing the quantitative phenotypes. See text for a definition of the seven parameters in the model (*V*, *u*, *q*, *t*, *d*, *H*, and *Z*).

The transmission pattern from parents to offspring can be tested in the unified mixed model to verify that the gene is segregating according to Mendelian expectations. The transmission pattern is characterized by three parameters: τ_1 is the probability that an *AA* individual transmits allele *A* to the offspring; τ_2 is the probability that *Aa* transmits *A*; and τ_3 is the probability that *aa* transmits *A*. Under Mendelian expectations, $\tau_1 = 1$, $\tau_2 = ½$, $\tau_3 = 0$, and no transmission of the major effect is obtained when the three τ values are equal. To infer a major gene, three conditions are usually required: 1) rejection of the no major effect hypothesis ($d = t = q = 0$); 2) failure to reject the hypothesis of Mendelian transmission; and 3) rejection of the hypothesis of no transmission of the major effect (equal τs model). Finally, maximum likelihood methods were used for fitting the models to data, and likelihood ratio tests were used for evaluating nested hypotheses.