# Familiality of Physical and Metabolic Characteristics That Predict the Development of Non–Insulin-Dependent Diabetes Mellitus in Pima Indians

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## Summary

**Susceptibility to non–insulin-dependent diabetes mellitus (NIDDM) is largely genetically determined. In Pima Indians, obesity, insulin resistance, and a low acute insulin response (AIR) to an intravenous glucose infusion are each predictors of the disease. To ascertain whether these phenotypes are genetically determined, we estimated their familiality in nondiabetic Pima Indians with a maximum-likelihood method. Percentage body fat (PFAT) was highly familial ($h^2$ =.76), whereas waist/thigh circumference ratio (W/T ratio) was not significantly familial after controlling for PFAT ($h^2$ = .16). AIR was also highly familial ($h^2$ = .80 at 10 min), even after controlling for PFAT and insulin action ($h^2$ = .70). Insulin action at physiologic plasma insulin concentrations was familial ($h^2$ = .61) but less so after controlling for PFAT and W/T ratio ($h^2$ = .38). At maximally stimulating insulin concentrations, insulin action was familial ($h^2$ = .45) and was less influenced by controlling for PFAT and W/T ratio ($h^2$ = .49). We conclude that in Pima Indians (1) PFAT and AIR are highly familial traits, (2) central distribution of fat is not a familial trait when controlled for PFAT, (3) 38%–49% of the variance in insulin action, independent of the effect of obesity, is familial, and (4) PFAT, AIR, and insulin action are useful traits to study genetic susceptibility to NIDDM. Because genetic parameter estimates are applicable only to the populations from which they were estimated, it is important to determine whether these estimates of familialities in Pima Indians can be confirmed in other populations before the utility of these traits in searching for NIDDM susceptibility genes in those populations can be fully advocated.**

## Introduction

Twin studies have shown convincingly that susceptibility to develop non–insulin-dependent diabetes mellitus (NIDDM) is largely genetically determined (Newman et al. 1987; Kaprio et al. 1992). In Pima Indians, a population with the highest reported prevalence of the disease, persons with NIDDM are characterized by obesity, particularly central obesity, insulin resistance, a low or absent acute insulin response (AIR) to an intravenous glucose infusion, and excess hepatic glucose production (Bogardus 1996). Prospective studies of this population have shown that all but the last of these metabolic phenotypes are independently predictive of the disease (Lillioja et al. 1993). We have inferred from these data that genes determining one or more of these phenotypes are likely to be genes that increase susceptibility to NIDDM.

Several studies of nondiabetic Pimas have indicated that obesity, insulin resistance, and AIR are familial traits (Lillioja et al. 1987; Knowler et al. 1991; Janssen et al. 1994), each of which may therefore have genetic determinants. Insulin action is distributed trimodally in the population, consistent with a codominant effect of an autosomally inherited gene (Bogardus et al. 1989), and a mutation in the gene encoding the intestinal fatty-acid binding protein is associated with a small decrease in insulin sensitivity (Baier et al. 1995). In addition, a marker on chromosome 6p, near the gene encoding tumor necrosis factor alpha, is linked to obesity in the Pimas (Norman et al. 1995), and an anonymous marker on chromosome 1p is linked to the AIR (Thompson et al. 1995). These data do not prove, but are consistent with the hypothesis, that obesity, insulin resistance, and AIR are genetically determined phenotypes. To test this hypothesis further, we have estimated the familiality of each of these prediabetic traits in nondiabetic Pimas by use of a maximum-likelihood method. The results indicate that percentage body fat (PFAT), AIR, and insulin action are familial traits to varying extents in the Pimas and, therefore, may be useful phenotypes to study to identify NIDDM susceptibility genes in this population.

## Subjects, Material, and Methods

### *Subjects and Clinical Testing*

The volunteers for this study are members of the Gila River Indian Community in Arizona, who are predomi-

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**Table 1**

**Number of Volunteers Used for Familiality Analyses**

| No. of Examinations | No. of Nondiabetic Volunteers | No. of Volunteers with Normal Glucose Tolerance and AIR Measurement |
|---|---|---|
| 1 | 509 | 275 |
| 2 | 253 | 146 |
| 3 | 181 | 114 |
| 4 | 135 | 83 |
| 5 | 103 | 64 |
| 6 | 66 | 40 |
| 7 | 55 | 33 |
| 8 | 42 | 24 |
| 9 | 28 | 14 |
| 10 | 12 | 5 |
| Total | 1,384 | 798 |

NOTE.—Data on all phenotypes were not completed on all volunteers at all examinations.

nantly of either Pima or the closely related Papago heritage. After informed consent, volunteers are admitted to the clinical research ward for ~7–15 d, during which time they eat a weight-maintaining diet and undergo a series of tests (described briefly below) to characterize their body composition and glucose homeostasis as described by Lillioja et al. (1993). Each volunteer is asked to return yearly to repeat the same tests. The study protocol was approved by the Institutional Review Board of the National Institutes of Health and the Tribal Council of the Gila River Indian Community.

The data used in the analysis came from 509 nondiabetic volunteers, 275 of whom had AIR measurements. Many of these subjects were examined on multiple occasions (table 1). Although the measurements most often were not available in parental generations, extended pedigrees were constructed to utilize all possible genetic relationships among close, as well as distant, relatives in familiality analyses. There were a total of 153 distinct pedigrees. The total numbers of first-, second-, and third-degree relative pairs, respectively, varied from 178, 175, and 12 to 584, 509, and 43, depending on the phenotype analyzed. Data on many of these subjects have been published previously (Lillioja et al. 1987, 1993; Bogardus et al. 1989).

Waist circumference was measured at the umbilicus with the volunteer supine, and the thigh circumference was measured at the gluteal fold with the volunteer standing. PFAT was determined by underwater weighing as described by Lillioja et al. (1993). A 75-g oral glucose-tolerance test was performed, and each volunteer's glucose-tolerance status was categorized according to the criteria of the World Health Organization (WHO) (WHO Study Group 1985).

Fasting plasma insulin concentrations were measured (Lillioja et al. 1993) on blood samples obtained on four separate mornings after an overnight fast. The AIR to glucose was determined after a 12-h overnight fast during and following a 3.6-min infusion of 25 g dextrose (Chen and Porte 1976). Blood samples were collected fasting and at 3, 4, 5, 6, 8, and 10 min. The insulin response was calculated as the increment over the fasting concentration at each time point. Analyses of results of this intravenous glucose-tolerance test were restricted further to volunteers' admission when they had normal glucose tolerance. This restriction was made under the assumption that lower insulin responses during this test may result secondarily from "glucotoxicity" or other unknown mechanisms as part of the transition from normal glucose tolerance to diabetes, when the insulin response is often absent.

On a separate day, insulin action was measured using a two-step, hyperinsulinemic, euglycemic clamp (at a plasma glucose concentration of ~100 mg/dl) as described by Lillioja et al. (1987). The lower rate of insulin infusion during the clamp resulted in a physiologic concentration (~130 μU/ml), and the high dose was chosen to estimate maximal insulin action in vivo (~2,000 μU/ml). Before and during the low-dose insulin infusion, tracer amounts of [3-$^3$H] glucose were infused to permit calculation of the rate of glucose disappearance (Steele 1959). The effects of variations in plasma glucose concentrations during the clamp study were adjusted to 100 mg/dl as suggested by Best et al. (1981). Differences between individual subjects in insulin concentrations during the low-dose insulin infusion were taken into account in the calculation of glucose uptake (Gottesman et al. 1983). Glucose uptake rates were normalized to metabolic body size, calculated as the free-fat body mass plus 17.7 kg, since metabolic rate is not directly proportional to fat-free body mass (Lillioja and Bogardus 1988).

### *Estimation of Familiality*

Familiality estimations were carried out with mixed models (fixed and random effects) using a restricted maximum-likelihood (REML) approach (Patterson and Thompson 1971; Harville 1977). A derivative-free method (Smith and Graser 1986) was used, which maximizes the log likelihood without calculating derivatives or expectations. Data from multiple visits were used, when available, to reduce the amount of variance due to unique individual environments that otherwise appear in the total phenotypic variance (Falconer 1985). This reduction of the phenotypic variance represents the gain in accuracy from multiple measurements, and therefore it increases estimates of the proportion of additive genetic variance in total phenotypic variance. All statistical models included sex as a fixed effect. Random effects

**Table 2**

**Familiality Estimates of Body Composition and Insulin Action in Nondiabetic Pimas**

| Phenotypes[a] | Covariates | $h^2 \pm$ SE |
|---|---|---|
| BMI | Age, sex | .49 ± .014 |
| PFAT | Age, sex | .76 ± .016 |
| Waist circumference | Age, sex | .81 ± .011 |
| Thigh circumference | Age, sex | .63 ± .022 |
| W/T | Age, sex | .39 ± .041 |
| | Age, sex, PFAT | .16 ± .056 |
| Fasting insulin (m/ml) | Age, sex | .65 ± .022 |
| | Age, sex, PFAT, W/T | .46 ± .029 |
| $M_{low}$ | Age, sex | .61 ± .049 |
| | Age, sex, PFAT, W/T | .38 ± .061 |
| $M_{high}$ | Age, sex | .45 ± .047 |
| | Age, sex, PFAT, W/T | .49 ± .063 |

NOTE.—Estimates of SEs were obtained by PAP, using converged values from MTDFREML. Parameter estimates from PAP were close to MTDFREML estimates, generally within the SEs shown. Familiality is a function of additive genetic and shared environmental effects.

[a] Rate of insulin-mediated glucose uptake measured during the hyperinsulinemic, euglycemic clamp at physiologic ($M_{low}$) or maximally stimulating ($M_{high}$) insulin concentrations.

included each individual's direct genetic effects as well as a nongenetic effect that accounted for multiple estimates for the same phenotype across time on each individual, when applicable. The covariates included in models varied, depending on the variable being analyzed, and for each subject included age at each measurement, PFAT, W/T ratio, and the rate of insulin-mediated glucose uptake measured during the hyperinsulinemic, euglycemic clamp. Two familial parameters ($\sigma_g^2$ and $\sigma_e^2$), as well as covariates, were estimated for each trait. Data were examined for normality and for outliers before each analysis. Natural logarithmic transformations were made when necessary to satisfy the assumptions of normality. Iterations were initiated using prior estimates of the parameters and were terminated when the convergence criterion (variance in simplex functional values of $<10^{-6}$ from two consecutive runs) was met. Iterations were then restarted using several different prior estimates to ensure that a "global" maximum is reached, i.e., all iterations eventually converge to the same estimates. A publicly available software (MTDFREML; Boldman et al. 1993), which implements the derivative-free REML approach, was used for data analyses. This software has been widely used in estimation of genetic and phenotypic parameters in animal and plant genetics with or without repeated measurements. Following is a brief discussion of the mixed-model methodology used in the analyses.

The general mixed model for a vector of observations is $\mathbf{y} = \mathbf{X\beta} + \mathbf{Zu} + e$ (Henderson 1950), where $\boldsymbol{\beta}$ = vector of fixed effects associated with records in y by X; $\mathbf{u}$ = vector of random effects associated with records in y by Z; and $e$ = residual effects.

The mixed model equations can then be written as:

$$\begin{bmatrix} \mathbf{X'R^{-1}X} & \mathbf{X'R^{-1}Z} \\ \mathbf{Z'R^{-1}X} & \mathbf{Z'R^{-1}Z + G^{-1}} \end{bmatrix} \begin{bmatrix} \hat{\boldsymbol{\beta}} \\ \hat{u} \end{bmatrix} = \begin{bmatrix} \mathbf{X'R^{-1}}y \\ \mathbf{Z'R^{-1}}y \end{bmatrix},$$

where $\mathbf{X}$ = design matrix corresponding to fixed effects; $\mathbf{Z}$ = design matrix corresponding to random effects; $\mathbf{R} = \mathbf{I}\sigma_g^2$, assumed to be diagonal so that calculations with $\mathbf{R}^{-1}$ are easy; and $\mathbf{G} = \mathbf{A}\sigma_g^2$, where $\sigma_g^2$ is the additive genetic variance and $\mathbf{A}$ is the relationship matrix, which includes all possible relationships among the individuals and has the size of $n \times n$, where $n$ is the total sample size.

Following solution of these equations through the iterative process, familiality estimates were derived from the estimated additive genetic and phenotypic variance parameters by use of the conventional formula $h^2 = \sigma_g^2/\sigma_p^2$ (Falconer 1985). However, it should be noted that the $h^2$ notation is used to report estimates of "familiality" rather than "heritability" throughout this paper, and, as such, it is a function of additive genetic effects as well as the confounding shared environmental effects. To confirm the REML familiality estimates and to obtain standard errors for these estimates, the converged values from MTDFREML were used as initial estimates in the pedigree analysis package (PAP; Hasstedt 1994) and parameters were re-estimated using exact likelihoods. The estimated parameter values produced by PAP were very close to those obtained using the derivative-free method, generally within the SEs shown in tables 2 and 3.

**Table 3**

**Familiality Estimates of the AIR in Nondiabetic Pimas**

| Phenotype | Covariates | $h^2 \pm$ SE |
|---|---|---|
| AIR at 3 min | Age, sex | .43 ± .031 |
| | Age, sex, % body fat, $M_{low}$ | .35 ± .030 |
| AIR at 4 min | Age, sex | .50 ± .030 |
| | Age, sex, % body fat, $M_{low}$ | .44 ± .031 |
| AIR at 5 min | Age, sex | .65 ± .026 |
| | Age, sex, % body fat, $M_{low}$ | .59 ± .032 |
| AIR at 6 min | Age, sex | .62 ± .022 |
| | Age, sex, % body fat, $M_{low}$ | .55 ± .029 |
| AIR at 8 min | Age, sex | .70 ± .020 |
| | Age, sex, % body fat, $M_{low}$ | .62 ± .029 |
| AIR at 10 min | Age, sex | .80 ± .020 |
| | Age, sex, % body fat, $M_{low}$ | .70 ± .028 |

NOTE.—AIRs were analyzed only from subjects with normal glucose tolerance. Familiality is a function of additive genetic and shared environmental effects.

## Results and Discussion

NIDDM is a complex disease that makes classical genetic approaches to identify its susceptibility genes very difficult at best. Dissection of NIDDM into genetically less complex phenotypes that are more amenable to genetic studies may therefore hasten progress to find its causative etiologic metabolic determinants. We have hypothesized that genes that determine one or more of previously identified phenotypes that predict the development of NIDDM (Lillioja et al. 1993) are likely to be genes that increase susceptibility to NIDDM. In this study, we have estimated the familiality of these phenotypes to ascertain which are potentially genetically determined and would therefore be suitable for further genetic studies to identify susceptibility genes for NIDDM.

The results indicate that each of the previously determined prediabetic phenotypes (Lillioja et al. 1993) have significant familial determinants. Body mass index (BMI), calculated as body weight (in kg) divided by height (in $m^2$), is a measure of the degree of overweight and was a familial trait ($h^2$ = .49). PFAT, an estimate of degree of obesity, i.e., relative excess of body lipid stores, was highly familial ($h^2$ = .76) in this generally obese population, as was the waist circumference ($h^2$ = .81) (table 2). The similarity of these latter findings was expected, since the waist circumference is highly correlated with PFAT in male and female Pimas (Pearson $r$ = .85, $P$ = .0001; and $r$ = .75, $P$ = .0001, respectively). The W/T ratio, an estimate of the central distribution of fat, was not significantly familial ($h^2$= .16) after controlling for PFAT. These results indicate that—at least in the Pimas, in whom obesity is more centrally distributed than in Caucasians (Lillioja et al. 1991)—the important familial trait in regard to body adiposity is PFAT rather than the distribution of fat.

The high familiality of PFAT in the Pimas ($h^2$ = .76) was similar to results in Caucasian twins, in which degree of overweight was estimated using the BMI. In U.S. male twins (Carmelli et al. 1994), the estimated heritability for BMI was .65, and in a Scandinavian study of male and female twins reared apart (Stunkard et al. 1990) 72% and 69%, respectively, of the variance in BMI was due to genetic factors. Slightly different results were reported in another study (Bouchard et al. 1988), where the "heritability" estimate was .25 for PFAT, estimated by underwater weighing, in families of French descent near Quebec City, Canada. The small difference between their results and those in the Pimas may be explained in part by (1) the lower frequency of obese subjects in the Canadian population as compared to the Pimas and (2) the confounding "shared" environmental effects included in the familiality estimates in the Pimas. In addition, the common environmental effects of ~30% reported by Bouchard et al. (1988) make their results more similar to those reported for the Pimas.

There have been two other studies of the heritability of distribution of body fat. The first was in the population near Quebec City, Canada (Borecki et al. 1995), in which 37% of the phenotypic variance in the trunk-to-extremity ratio of skinfolds, after controlling for total fat mass, was attributable to a recessive major gene. In a study of Caucasian male twins, Selby et al. (1989) reported a similar heritability of the ratio of the waist-to-hip circumferences ($h^2$ = .31) after controlling for BMI. These estimates are considerably higher than the familiality of the W/T ratio found after controlling for PFAT in the Pimas ($h^2$ = .16). This may be because obese Pimas have a more central distribution of fat compared to Caucasians (Lillioja and Bogardus 1988). Before adjusting for PFAT, the familiality of the W/T ratio was similar ($h^2$ = .39) to that found in Caucasians in Quebec when controlling for fat mass.

The estimated familiality for insulin action in the Pimas ranged from .61 at physiological plasma insulin concentrations ($M_{low}$) to .45 at maximally stimulating insulin concentrations ($M_{high}$) (table 2). However, after controlling for the important covariates, PFAT and W/T ratio, the familiality of $M_{low}$ was lower, whereas the familiality of $M_{high}$ was essentially unchanged. These findings were expected, since $M_{low}$ is more highly correlated with obesity than was $M_{high}$ (Lillioja et al. 1987). The familiality of fasting plasma insulin concentrations, a surrogate measure of insulin action in vivo, was similar to the results for $M_{low}$ and $M_{high}$ (table 2).

We are aware of no other reports of the familiality of insulin action. However, Schumacher et al. (1992) have reported evidence in Caucasian pedigrees in Utah of a major gene effect for fasting plasma insulin concentrations, a surrogate measure of insulin action. They found that 33% of the variance in fasting plasma insulin concentrations was due to a major autosomal locus and that 11% was due to polygenic inheritance after controlling for obesity as estimated by BMI. These findings were quite similar to the present results in the Pimas, where, after controlling for age, sex, PFAT, and W/T ratios, the familiality of $M_{low}$ was .38, $M_{high}$ was .49, and fasting plasma insulin concentration was .46.

The familiality of the AIR increased during the course of the response, and this finding was unexpected ($h^2$ of .43, .50, .65, .62, .70, and .80 at 3, 4, 5, 6, 8, and 10 min, respectively; table 3). These higher familiality estimates at the later part of the response remained high after also controlling for insulin action and PFAT. In the past, we have used the mean insulin increment over fasting concentrations at earlier time points, 3, 4, and 5 min, as the measure of insulin secretory responsiveness in both clinical (Lillioja et al. 1993) and genetic linkage studies (Thompson et al. 1995), since the insulin increments are maximal at these times. The reason for the increasing familiality with time is not clear, but it is not

because of a correlation of the insulin increments at later times with insulin resistance. After controlling for PFAT and insulin action, the familiality remained high ($h^2$ = .70) at 10 min into the test. We conclude that the AIR at the later times reflects pancreatic β-cell insulin secretory function and that this is a highly familial trait in Pimas.

In conclusion, PFAT, AIR, particularly at later times during the test, and insulin action are familial traits in nondiabetic Pima Indians. These metabolic traits are predictive of NIDDM, and they should be useful phenotypes for genetic studies of susceptibility genes for the disease in this population. However, estimates of genetic parameters are applicable only to the populations from which they were estimated. It is important to determine whether the high estimates of familiality in this population can be confirmed in other populations before the utility of these traits in searching for NIDDM susceptibility genes in those populations can be fully advocated.

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