Heritability of Insulin Secretion and Insulin Action in Women with Polycystic Ovary Syndrome and Their First Degree Relatives*

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ABSTRACT

Polycystic ovary syndrome (PCOS), one of the most common endocrine disorders of reproductive age women, is associated with an increased risk of type 2 diabetes mellitus. Defects in both insulin action and insulin secretion contribute to this predisposition to diabetes, but the extent to which these defects are heritable among PCOS families has not been examined.

In the present study we used the frequently sampled iv glucose tolerance test to quantitate insulin secretion (AIRg), insulin action (Si), and their product (AIRg×Si) among women with PCOS (n = 33) and their nondiabetic first degree relatives (n = 48). We then quantitated the heritability of these measures from familial correlations estimated within a genetic model.

Familial (spousal, ρMF; parent-offspring, ρPO; and sibling, ρSS) correlations were derived for log-transformed body mass index (BMI) as well as for AIRg, Si, and AIRg×Si, the latter three of which were adjusted for BMI. There was no evidence of significant heritability for either lnBMI or lnSi in these families. In contrast, the sibling correlation (ρSS = 0.74) for lnAIRg was highly significant (χ² = 7.65; 1 df; P = 0.006). In addition, the parameter quantitating insulin secretion in relation to insulin sensitivity [i.e. ln(AIRg × Si)] was significant among siblings (ρSS = 0.74; χ² = 4.32; 1 df; P = 0.04).

In summary, the results of the present study indicate that there is an heritable component to β-cell dysfunction in families of women with PCOS. We conclude that heritability of β-cell dysfunction is likely to be a significant factor in the predisposition to diabetes in PCOS. (J Clin Endocrinol Metab 86: 2027–2031, 2001)

POLYCYSTIC OVARY SYNDROME (PCOS) affects between 4–8% of reproductive age women (1, 2), thus placing it among the most common endocrine disorders in this age group. In addition to its reproductive sequelae, PCOS is associated with an increased risk of developing type 2 diabetes, often at an early age (3–5).

Insulin resistance plays a key role in the predisposition to diabetes in PCOS (6, 7), but although a substantial proportion of insulin-resistant women with PCOS develops either impaired glucose tolerance or diabetes, this is not the case for most. In our previous studies we sought to identify factors that distinguish insulin-resistant women with PCOS and glucose intolerance from those who are able to maintain normoglycemia. We (8) as well as others (9) found that a proportion of nondiabetic women with PCOS had defects in insulin secretion, particularly when analyzed in relation to the ambient level of insulin resistance. Further, such defects were most evident among those women who had a first degree relative with type 2 diabetes (8). This latter finding suggested that there was a genetic contribution to the reduction in the ability of the β-cell to adequately compensate for insulin resistance, consistent with studies in nondiabetic family members of type 2 diabetics (10).

Given these findings, we hypothesized that heritability of defects in insulin secretion and/or insulin action would be evident within families of women with PCOS. We have tested this hypothesis in the present study using the frequently sampled iv glucose tolerance test (IVGTT) to simultaneously quantitate insulin secretion, insulin action, and their interrelationship among women with PCOS and their first degree relatives. The heritability of these measures was then determined from familial correlations estimated within a genetic model.

Subjects and Methods

Subjects with PCOS

Women with PCOS, 18–40 yr of age, were recruited from the Endocrinology Clinics of the University of Chicago between 1997 and 1999. All studies were approved by the institutional review board of the University of Chicago, and written informed consent was obtained from each subject.

A diagnosis of PCOS was assigned if subjects had historical, physical examination, and hormonal evidence of androgen excess and met the most commonly used diagnostic criteria for PCOS, often referred to as the NIH consensus criteria (11). Specifically, all had a history of oligo/amenorrhea, infertility, hirsutism, acne, or androgenetic alopecia and hyperandrogenemia, defined by a supranormal plasma free testosterone level (≥34.7 pmol/L) (12). Hormonal evidence of ovarian androgen overproduction was confirmed by an abnormal 17-hydroxyprogesterone response to GnRH agonist administration (12) or a supranormal plasma free testosterone level after administration of dexamethasone (12). Subjects with nonclassical 21-hydroxylase deficiency congenital adrenal hyperplasia, Cushing’s syndrome, and hyperprolactinemia were excluded from the study as were those known to be diabetic. All steroid preparations (including oral contraceptives) or medications...
known to alter insulin secretion and/or action had been discontinued for at least 2 months before screening and enrollment.

First degree relatives of subjects with PCOS

All available nondiabetic first degree relatives of women with PCOS were contacted and invited to participate in the study. Relatives were recruited without regard to the glucose tolerance status of the proband.

Characterization of insulin secretion and insulin sensitivity: frequently sampled IVGTT

Subjects were admitted after an overnight fast. Two iv catheters were placed, one for the administration of glucose and tolbutamide, and the other for blood drawing. Blood samples were drawn for glucose and insulin at −20, −15, −10, and 0 min, at which time 300 mg/kg glucose was administered as an iv bolus. Blood samples for glucose and insulin were obtained at 2, 3, 4, 5, 6, 8, 10, 12, 15, and 19 min. At 20 min, tolbutamide (125 mg/m²; Orionase, Upjohn, Kalamazoo, MI) was given iv. Thereafter, blood was sampled at 21, 22, 24, 26, 28, 30, 35, 40, 45, 50, 55, 60, 70, 80, 100, 120, 140, 180, 210, and 240 min.

Summary measures derived from the IVGTT included 1) first phase insulin secretion (AIRg) in response to glucose, calculated as the mean increment above basal of insulin values measured at 2, 3, 4, 5, 6, 8, and 10 min; 2) insulin sensitivity index (Si), calculated using the MINMOD program, as previously described (8), provided by Dr. R. N. Bergman (the insulin sensitivity index represents the increase in net fractional glucose clearance rate per unit change in plasma insulin concentration after the iv glucose load); and 3) the relationship between the acute insulin response to glucose (AIRg) relative to the degree of insulin resistance (Si). This relationship, referred to as the disposition index, is calculated as the product of Si and AIRg and provides a measure of β-cell secretory function adjusted for insulin sensitivity.

Assay methods

Plasma glucose was measured immediately using a glucose analyzer (model 2300 STAT, YSI, Inc., Yellow Springs, OH). The coefficient of variation of this method is less than 2%. Glycosylated hemoglobin was measured by boronate affinity chromatography with an intraassay coefficient of variation of 4% (Bio-Rad Laboratories, Inc., Hercules, CA). Serum insulin was assayed by a double antibody technique (4) with a lower limit of sensitivity of 20 pmol/L and an average intraassay coefficient of variation of less than 2%. The cross-reactivity of proinsulin in the RIA for insulin is approximately 40%.

Plasma testosterone was measured using a kit from Diagnostic Products (Los Angeles, CA). The free fraction of plasma testosterone and the concentration of sex hormone-binding globulin were measured by a competitive protein binding assay (4). The intra- and interassay coefficients of variation were 3.8% and 8.7%, respectively.

Data analysis/statistics

Phenotypic measures [body mass index (BMI), Si, AIRg, and AIRg × Si] were log-transformed to normalize their distributions and were also adjusted for any significant covariates found in this dataset. Covariates for each phenotype were tested for significance using a linear regression procedure in SAS statistical software (13).

Age, sex, and race were significant predictors for BMI in these families; thus, a residual was created adjusting for these factors and was used in all analyses. BMI, in turn, was the only covariate that was a significant predictor for lnAIRg, lnSi, and ln(AIRg × Si). These three measures were therefore adjusted for BMI in all analyses.

Spousal, sibling, and parent-offspring correlations were estimated from the covariate-adjusted residuals for each phenotype in the context of a genetic model provided by the REGC program in SAGE (14). Familial patterns of correlations were examined using class D regressive models, assuming no major gene effect (15, 16). In regressive models, genetic components of a trait can be estimated independently from related individuals because they successively condition each individual’s trait upon those of their ancestors. Class D regressive models are a specific type of regressive model that assumes that the
sibling correlations within a family are equal and not necessarily due solely to common parentage. Because our families were ascertained through PCOS probands, who have an increased risk for diabetes (3–5), an ascertainment correlation was employed where each family's likelihood was made conditional on the diabetes-related phenotype of the proband.

To test the significance of each familial correlation for a phenotype, the likelihood scores between nested models were compared. First, a general model that simultaneously estimated all three correlations (spousal, $r_{	ext{MF}}$; parent-offspring, $r_{	ext{PO}}$; and sibling, $r_{	ext{SS}}$) along with a population mean and variance was computed. Then a model fixing the spousal correlation parameter at zero (no spousal correlation model) was estimated and compared with the general model to assess the significance of the spousal correlation. If the spousal correlation was not significantly different from zero, a model fixing the spousal and the parent-offspring correlation parameters at zero (no parent-offspring model) was computed. This model was then compared with the no spousal correlation model to assess the significance of the parent-offspring correlation. Similarly, a model fixing the spousal and sibling correlation parameters at zero (no sibling correlation model) was generated and compared with the no spousal model to determine the significance of the sibling correlation. Finally, a model simultaneously fixing all three familial correlation parameters at zero was computed (no correlation model) to test the significance of all three correlations together.

Likelihood ratio tests (where twice the difference between ln likelihoods for nested models is asymptotically distributed as a $\chi^2$) were used to compute a $\chi^2$ statistic for each correlation tested and its corresponding $P$ value. The number of degrees of freedom for this $\chi^2$ statistic is equal to the difference in the number of independently estimated parameters between the two models.

**Results**

**Baseline measures**

Baseline clinical and hormonal measures for PCOS subjects and their first degree relatives are shown in Tables 1 and 2, respectively. Of the 48 first degree relatives in this study, 31 (65%) were Caucasian, 12 (25%) were African-American, 4 (8%) were Asian, and 1 (2%) was Hispanic. Sixty-two percent of the first degree relatives were female, and 38% were male. As expected, the PCOS subjects had fasting hyperinsulinemia and substantially elevated levels of total and free testosterone. The mean glycohemoglobin level was normal in both PCOS subjects and relatives.

**Familial correlations**

Familial correlations were estimated for the natural log of BMI, Si, AIRg, and AIRg × Si for 17 informative families with an average family size of 2.5. Table 3 shows the parameter estimates from a regressive model assessing the familial correlations for the lnBMI residual. Even though the spousal correlation ($r_{\text{MF}} = 0.42$) was the strongest correlation estimated from the general model, this estimate was not significantly different from zero ($\chi^2 = 1.97; 1 \text{ df}; P = 0.16$). Both the parent-offspring and sibling correlations for lnBMI were even weaker and therefore were not significant ($r_{\text{PO}} = 0.17; \chi^2 = 0.98; 1 \text{ df}; P = 0.32; r_{\text{SS}} = 0.10; \chi^2 = 0.25; 1 \text{ df}; P = 0.62$). These results suggest that BMI is not highly familial in these PCOS families.

Correlations for lnSi, even after adjustment for BMI, were also not significant (Table 4). Both the spousal correlation for lnSi ($r_{\text{MF}} = -0.01$) and the parent-offspring correlation ($r_{\text{PO}} = 0.08$) from the general model were not significantly different from zero ($\chi^2 = 0.002; 1 \text{ df}; P = 0.96$ and $\chi^2 = 0.09; 1$
df; $P = 0.76$, respectively). The sibling correlation for $\ln Si$ was estimated at its lower bound of zero.

Table 5 shows the parameter estimates from a regressive model assessing the familial correlations for $\ln AIRg$ adjusted for BMI. From the general model, the spousal correlation ($r_{MF}$) was $0.19$, which was not significantly different from zero ($\chi^2 = 0.06; 1$ df; $P = 0.81$). The parent-offspring correlation ($r_{PO}$) was $0.23$, which was also not significantly different from zero ($\chi^2 = 0.95; 1$ df; $P = 0.33$). In contrast, the sibling correlation ($r_{SS} = 0.74$) was highly significant ($\chi^2 = 7.65; 1$ df; $P = 0.006$).

Table 6 displays the familial correlations for the log-transformed disposition index, $\ln(AIRg \times Si)$. The spousal correlation ($r_{MF} = 0.41$) was not significantly different from zero ($\chi^2 = 1.33; 1$ df; $P = 0.25$), which was also the case for the parent-offspring correlation ($r_{PO} = 0.48; \chi^2 = 1.84; 1$ df; $P = 0.18$). The sibling correlation ($r_{SS}$), however, was $0.74$, which was similar to that for $\ln AIRg$ alone and also statistically significant ($\chi^2 = 4.32; 1$ df; $P = 0.04$).

### Discussion

Women with PCOS are at substantial risk for development of impaired glucose tolerance and type 2 diabetes mellitus (4, 5). Although it is well established that profound reductions in insulin sensitivity antedate the development of diabetes in PCOS (6, 7), more recently it has been recognized that insulin secretory dysfunction may also be present early in the evolution of glucose intolerance in these women (1, 9). Further, the alterations in insulin secretion appear to be particularly evident among those PCOS women who have a first degree relative with diabetes (1).

In the present study we used the rapidly sampled iv glucose tolerance test to quantitate insulin sensitivity and insulin secretion with the aim of determining whether either or both are heritable traits in PCOS families. Among the PCOS families studied, there was a significant familial (sibling) correlation for the acute insulin response to iv glucose and a lesser, but still significant, correlation when this measure was related to the degree of insulin resistance in the form of
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Their product, the disposition index (AIRG × Si). These results provide evidence that β-cell function is heritable in PCOS families and are consistent with recent studies by Elbein et al. (10), who found evidence for heritability of these measures in nondiabetic family members of type 2 diabetics. Our results also indicate that spousal correlations for AIRg and AIRg × Si were not significant. This implies that a shared environment does not have a significant role in predicting β-cell function and, along with a significant sibling correlation, is consistent with a genetic model of inheritance.

Contrary to what was expected as well as previously reported (17), we did not find evidence for heritability of insulin resistance in PCOS families. Our ability to assess the heritability of insulin sensitivity in PCOS families, however, was limited. A relatively small number of subjects was studied, many of whom were both obese and profoundly insulin resistant, thus limiting the variability of this measure (18).

Given that BMI has shown reasonably strong heritabilities in other studies (19, 20), it is interesting to note that BMI did not appear familial in these kindreds. Because BMI largely determines and is highly correlated with insulin sensitivity, our results showing lack of heritability for BMI and the insulin sensitivity index even when adjusted for BMI, are consistent with one another.

In conclusion, the results of the present study indicate that there is an heritable component to β-cell dysfunction in families of women with PCOS. This heritability of β-cell dysfunction is likely a significant factor in the predisposition to diabetes in PCOS.

References
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