Changes in Insulin Sensitivity in Response to Troglitazone Do Not Differ Between Subjects With and Without the Common, Functional Pro12Ala Peroxisome Proliferator-Activated Receptor-γ2 Gene Variant

Results from the Troglitazone in Prevention of Diabetes (TRIPOD) study

OBJECTIVE — We have tested whether the Pro12Ala variant of the peroxisome proliferator–activated receptor (PPAR)-γ nuclear receptor involved in thiazolidinedione (TZD) action accounted for the failure of troglitazone to increase insulin sensitivity in nondiabetic Hispanic women with previous gestational diabetes treated in the Troglitazone in Prevention of Diabetes (TRIPOD) study.

RESEARCH DESIGN AND METHODS — Ninety-three women assigned to troglitazone had intravenous glucose tolerance tests at randomization and after 3 months of treatment with troglitazone, 400 mg/day, and were genotyped for the Pro12Ala variant of the PPAR-γ gene. Subjects were divided into tertiles based on their change in minimal model insulin sensitivity (Si) during the first 3 months of troglitazone treatment.

RESULTS — The mean changes in Si in the bottom, middle, and top tertiles of Si response were 0.21 ± 0.57, 0.40 ± 0.26, and 2.36 ± 1.30 min⁻¹ per μU/ml • 10⁻⁴, respectively. Frequencies of the Ala/Ala genotype were 30, 22, and 26% in the same tertiles (P = 0.77). Analysis of phenotypes by genotype revealed only small differences between the Pro/Pro and Ala/Ala groups, respectively, in baseline Si (2.76 ± 0.19 vs. 2.33 ± 0.33 × 10⁻⁴ min⁻¹ per μU/ml; P = 0.27), the change in Si after 3 months of troglitazone treatment (1.19 ± 0.17 vs. 0.93 ± 0.30; P = 0.46), and the cumulative incidence of diabetes during a median follow-up of 30 months (13 vs. 17%; P = 0.66).

CONCLUSIONS — Among young Hispanic women at high risk for type 2 diabetes, the Pro12Ala variant of the PPAR-γ receptor gene did not explain the failure of ~1/3 of subjects to increase their insulin sensitivity when placed on troglitazone at a dose of 400 mg/day.

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Response to TZDs and Pro12Ala PPAR-\(\gamma\)2 variant

sensitization is not expected to lower glucose levels to an important degree in such patients. However, studies in which direct measurements of insulin sensitivity have been made reveal that some individuals do not experience an increase in insulin sensitivity when they are exposed to a TZD (11,12). In the Troglitazone in Prevention of Diabetes (TRIPOD) study (12), nondiabetic women with recent gestational diabetes whose change in insulin sensitivity during the first 3 months of troglitazone treatment was in the lowest tertile for treated subjects had a mean change in \(S_i\) that was slightly less than zero and similar to placebo-treated subjects. Clinical and metabolic characteristics at baseline and compliance with study medications were not useful in distinguishing these “nonresponders” from women whose insulin sensitivity increased in response to the drug (12). For the present report, we genotyped women in the troglitazone arm of the TRIPOD study to determine whether the Ala variant at the 12 position of the PPAR-\(\gamma\) receptor gene accounted for the lack of response to the insulin-sensitizing effects of troglitazone. As a secondary focus, we tested the effects of the Ala variant on several physiologic variables and on the risk of diabetes during troglitazone treatment.

**RESEARCH DESIGN AND METHODS** — The rationale, methods, and results from TRIPOD have previously been presented (12,13). Briefly, subjects were recruited from Los Angeles County Women’s and Children’s Hospital. Pivotal eligibility criteria were Hispanic ethnicity, previous gestational diabetes, and a sum of five oral glucose tolerance test plasma glucose concentrations \(\geq 625 \text{ mg/dl} (34.7 \text{ mmol/l})\), predicting a 70% risk of diabetes in the next 5 years (14). Women were randomized to placebo or troglitazone (400 mg/day), which was administered in a double-blind fashion. Enrollment continued until 266 subjects were randomized. The study protocol included a tolbutamide-modified, frequently sampled intravenous glucose tolerance test (15) performed at baseline and repeated after 3 months of treatment. The subjects were maintained on the drug for a median of 30 months after randomization and tested for diabetes with fasting glucose levels every 3 months and 75-g oral glucose tolerance tests (16) annually.

**Genotyping**

Women who completed the baseline and 3-month intravenous glucose tolerance tests were genotyped for the Pro12Ala polymorphism by the PCR restriction-fragment–length polymorphism technique. In addition to 93 subjects randomized to troglitazone, genotypes were available on 55 subjects randomized to placebo. The DNA was spun down in a 96-well plate and amplified with standard reaction and cycling conditions in 50-\(\mu\)l reactions containing PCR buffer, 10 mmol dNTPs, 12.5 pmol sense primer (5’-GCGAATTCAGCCCGTTCG-3’), 12.5 pmol antisense primer (5’-GATATGTTCAGACAGTGGATCAGTAGAAG GAACTCGTCTTCG-3’), and 1 unit of Taq polymerase (Gibco, Carlsbad, CA). An aliquot of the amplified DNA was subjected to electrophoresis through a 3% agarose gel, stained with ethidium bromide, and DNA visualized by ultraviolet transillumination. DNA product sizes for the PPAR-\(\gamma\)2 genotypes were: Pro/Pro, 270; Pro/Ala, 23 Ala/Pro, and 1 Ala/Ala troglitazone-treated subject, yielding genotype frequencies of 0.87 (Pro) and 0.13 (Ala). Genotype distribution did not deviate significantly from Hardy-Weinberg equilibrium. The 23 Ala/Pro and 1 Ala/Ala subjects were collapsed into one Ala/− group to facilitate statistical analysis.

The Pro/Pro and Ala/− groups did not differ significantly in terms of age (Pro/Pro, 35 ± 7; and Ala/−, 34 ± 7 years; \(P = 0.29\)), BMI (Pro/Pro, 31 ± 6, and Ala/−, 30 ± 5 kg/m\(^2\); \(P = 0.88\)), or fasting glucose at entry (Pro/Pro, 95 ± 11, and Ala/− 93 ± 13 mg/dl; \(P = 0.54\)). MinMOD values also did not differ significantly between the two groups at entry (Pro/Pro, 2.76 ± 0.19, and Ala/−, 2.33 ± 0.33 min\(^{-1}\) per \(\mu\)U/ml × 10\(^{-4}\); \(P = 0.27\)).

**Response tertiles**

Subjects were divided into response tertiles based on their change in \(S_i\) after 3 months of treatment. The mean changes in \(S_i\) in the bottom, middle, and top response tertiles were −0.21 ± 0.57, 0.91 ± 0.26, and 2.58 ± 1.32 min\(^{-1}\) per \(\mu\)U/ml × 10\(^{-4}\), respectively. Among the three tertiles, there was no trend for baseline \(S_i\), which averaged 2.84 ± 1.78, 2.05 ± 1.23, and 3.02 ± 2.50 min\(^{-1}\) per \(\mu\)U/ml × 10\(^{-4}\) in the bottom, middle, and top response tertiles, respectively. A response tertile × genotype \(\chi^2\) test did not deviate significantly from the values expected in the absence of a genotype effect (\(P = 0.77\)) (Table 1). Of note, the response of the single Ala/Ala individual was in the middle tertile, with a \(\delta\) value of 0.87 min\(^{-1}\) per \(\mu\)U/ml × 10\(^{-4}\).

**Development of diabetes**

During the subsequent period, during which subjects were maintained on troglitazone (median duration 30 months), the cumulative incidence of diabetes among those treated with troglitazone did not differ significantly by genotype and was...
reduced to a similar extent compared with placebo in the two genotypes (Fig. 1).

Continuous variables
The post hoc power calculation showed that we had 80% power to detect effects on continuous variables >0.67 times the SD of the measured variable. $S_i$ increased to a similar extent in the two groups after 3 months of troglitazone treatment (Pro/Pro, 1.19 ± 0.17, and Ala/−, 0.93 ± 0.30 min$^{-1}$ per μU/ml · 10$^{-4}$; P = 0.46). Furthermore, the Pro12Ala genotype did not predict change in the following values: fasting glucose (Pro/Pro, −3.9 ± 1.0, and Ala/−, −2.2 ± 1.6 mg/dl; P = 0.35), fasting insulin (Pro/Pro, −5.9 ± 8.5, and Ala/−, −2.5 ± 8.6 μU/ml; P = 0.17), acute insulin response (Pro/Pro, −60.3 ± 25.9, and Ala/−, 5.4 ± 41.2 mg/dl; P = 0.17), and disposition index (Pro/Pro, 297 ± 74, and Ala/−, 389 ± 125; P = 0.53). There were no significant changes in body weight over the 3-month period in either genotype group. Genotype did not influence changes in total cholesterol, HDL cholesterol, or triglycerides over the 3-month period (all P ≥ 0.23).

CONCLUSIONS — We divided Hispanic women at high risk for type 2 diabetes into tertiles based on their change in insulin sensitivity during 3 months of troglitazone treatment. Women in the lowest tertile had an average response that was less than zero and, accordingly, represent women who failed to respond to the insulin-sensitizing effect of troglitazone at 400 mg/day. We previously reported that those women did not enjoy protection from type 2 diabetes during the TRIPOD study. The frequency of the common, functional Pro12Ala variant in the PPAR-γ2 gene in troglitazone nonresponders was very similar to the frequency of the variant in women who responded to the drug with modest (middle tertile) and robust (highest tertile) increases in $S_i$. This observation provides strong evidence that the Pro12Ala variant did not account for the prevalence of troglitazone nonresponders in the TRIPOD cohort. Additional analyses of continuous variables revealed a numerically smaller increase in $S_i$ in the Ala/− subjects than in the Pro/Pro subjects, but this difference did not approach standard levels of statistical significance in this relatively small cohort. Just as important, it was very small in magnitude. The troglitazone-attendant reduction in the cumulative incidence of diabetes was similar in the two genotypes. Thus, although we cannot exclude an impact of the Pro12Ala variant on clinical or metabolic responses to troglitazone, our data reveal that any such effect must be very small in Hispanic women with prior gestational diabetes.

Analogous to this study, Bluher et al. (17) failed to demonstrate effects of the Ala/− variant on the fasting glucose and HbA1c responses to pioglitazone among diabetic subjects. Because an improvement in insulin action does not guarantee a clinically significant improvement in fasting glucose and HbA1c, it is not surprising that the nonresponse rate by the definition of Bluher et al. was higher than in the present study, which defines nonresponse mechanistically based on insulin action. However, both Bluher et al. and we appear at odds with in vitro studies (9,10) that showed reduced binding of the Ala protein to the PPAR-responsive element of several genes and decreased transactivation in response to rosiglitazone. This in vivo/in vitro discrepancy for TZD effects is probably not due to the use of different agents in vivo and in vitro because no differences in response rates (glucose lowering) were observed among troglitazone, pioglitazone, and rosiglitazone in 127 type 2 diabetic patients who were randomly assigned to treatment with pioglitazone or rosiglitazone when troglitazone was withdrawn from clinical use (18).

What other mechanisms might account for nonresponse to troglitazone? Here we can only speculate. One possibility is that Pro12Ala is only one of a host of genetic and environmental factors influencing response. Possible additional modifiers of response to TZDs are other genetic variants, not only in PPAR-γ2, but also in numerous associated molecules. A short list of candidates includes the retinoid X receptor-α (RXR-α), the PPAR-γ co-activator-1, lipoprotein lipase, muscle

Table 1 — Mean change in $S_i$ in response to 3 months of troglitazone treatment and corresponding genotype frequencies in women grouped into tertiles of $S_i$ change

<table>
<thead>
<tr>
<th>Tertile</th>
<th>Change in $S_i$*</th>
<th>Pro/Pro (%)</th>
<th>Ala/− (%)</th>
<th>Total (%)</th>
<th>Pro/Pro (%)</th>
<th>Ala/− (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottom tertile</td>
<td>−0.21 ± 0.57</td>
<td>21 (70)</td>
<td>9 (30)</td>
<td>30</td>
<td></td>
<td></td>
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<tr>
<td>Middle tertile</td>
<td>0.91 ± 0.26</td>
<td>25 (78)</td>
<td>7 (22)</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Top tertile</td>
<td>2.58 ± 1.32</td>
<td>23 (74)</td>
<td>8 (26)</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cohort</td>
<td>1.11 ± 1.41</td>
<td>69 (74)</td>
<td>24 (26)</td>
<td>93</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are n (%) of women with each genotype or means ± SD. Observed genotype frequencies did not differ significantly from expected frequencies (P = 0.77 by χ$^2$ test). *(3-month $S_i$) — (baseline $S_i$). $S_i$ is insulin sensitivity calculated with the minimal model (in min$^{-1}$ per μU/ml · 10$^{-4}$). †The tertiles are of unequal size because the cutoff points are based on all troglitazone-treated subjects (12), including those whose genotyping failed for technical reasons.

![Cumulative incidence of diabetes](image)
carnitine palmitoyltransferase-1, fatty acid-binding protein, and CCAAT/enhancer-binding protein α. None of these were assessed in the present study. Variability in TZD metabolism seems less likely because estimators of systemic troglitazone exposure in clinical trials do not improve the prediction of response beyond that obtained based on knowledge of the administered dose (19). The clinical state of the patient, including fasting plasma glucose, HbA1c, BMI, and fasting C-peptide, may further confound any effects of the Pro12Ala variant to an extent that statistical adjustments can only partially address (17).

Our study sample did not replicate previous reports associating the Ala/– variant with a higher insulin sensitivity, higher BMI, and protection against the development of diabetes. Although we can provide no definitive explanation for this difference, it is important to note that the TRIPOD cohort was highly unrepresentative of the population as a whole because the cohort was selected for gestational diabetes and relatively high glucose levels. Thus, effects of the Ala variant that have been observed across a range of phenotypes in the population as a whole could have been absent due to the narrow inclusion criteria.

In conclusion, we observed no evidence that isolated knowledge of a subject’s Pro12Ala PPAR-γ2 genotype is useful to identify nonresponders to the insulin-sensitizing effect of troglitazone treatment in young, high-risk Hispanic women. Furthermore, while we cannot fully exclude effects of the Ala variant on insulin sensitivity or the risk of type 2 diabetes in these women, any such effects must be very small and of limited clinical significance. Thus, we conclude that isolated assessment of the Pro12Ala genotype has no apparent clinical utility in the identification of high-risk Hispanic women who will not benefit from TZD treatment.

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