

Sequence Variation in *PPARG* May Underlie Differential Response to Troglitazone

Johanna K. Wolford,¹ Kimberly A. Yeatts,¹ Sharanjeet K. Dhanjal,² Mary Helen Black,² Anny H. Xiang,² Thomas A. Buchanan,³ and Richard M. Watanabe²

Thiazolidinediones (TZDs) are peroxisome proliferator-activated receptor- γ (PPARG) agonists used to treat type 2 diabetes. TZDs can also be used to reduce rates of type 2 diabetes in at-risk individuals. However, a large fraction of TZD-treated patients (30–40%) do not respond to TZD treatment with an improvement in insulin sensitivity (S_i). We hypothesized that variation within the gene encoding *PPARG* may underlie this differential response to TZD therapy. We screened ~40 kb of *PPARG* in 93 nondiabetic Hispanic women (63 responders and 30 nonresponders) with previous gestational diabetes who had participated in the Troglitazone In the Prevention Of Diabetes study. TZD nonresponse was defined as the lower tertile in change in S_i after 3 months of treatment. Baseline demographic and clinical measures were not different between responders and nonresponders. We identified and genotyped 131 variants including 126 single nucleotide polymorphisms and 5 insertion-deletion polymorphisms. Linkage disequilibrium analysis identified five haplotype blocks. Eight variants were associated with TZD response ($P < 0.05$). Three variants were also associated with changes in S_i as a continuous variable. Our results suggest that *PPARG* variation may underlie response to TZD therapy in women at risk for type 2 diabetes. *Diabetes* 54:3319–3325, 2005

The thiazolidinedione (TZD) class of insulin-sensitizing drugs are agonists for the nuclear receptor peroxisome proliferator-activated receptor- γ (PPARG) and widely used to treat type 2 diabetes (1–4). There is mounting evidence that TZD therapy can be used in people at high risk of type 2 diabetes to improve metabolic parameters (5–7) or possibly prevent (8) type 2 diabetes. An example is the Troglitazone in the

Prevention of Diabetes (TRIPOD) study, a placebo-controlled intervention trial that compared the effects of troglitazone and placebo on type 2 diabetes rates, S_i , and β -cell function in Hispanic women with previous gestational diabetes (8–10). In TRIPOD, type 2 diabetes incidence was reduced 55% in women who received troglitazone, and protection from type 2 diabetes persisted for at least 8 months following discontinuation of treatment despite the return of insulin resistance. These findings suggest that TZDs may have significant value in delaying or preventing type 2 diabetes in high-risk individuals.

A large fraction of individuals, both with type 2 diabetes (1,2,11,12) or who are at risk for type 2 diabetes (5,8,10), do not respond to TZD therapy. In individuals with type 2 diabetes, nonresponse has not been carefully characterized, but data from studies in at-risk individuals suggests that a lack of improvement in insulin sensitivity (S_i) may account for the lack of response to TZD therapy (6,8). In the Troglitazone In the Prevention Of Diabetes (TRIPOD) study, ~30% of treated women did not show an improvement in S_i (8); they gained no protection from type 2 diabetes when compared with the placebo group. Assessment of baseline clinical and physiologic measurements revealed similar levels of adiposity, fasting glucose and insulin, S_i and β -cell function, fasting lipids, contraceptive use, and compliance with study medication between responders and nonresponders, suggesting that these measures do not predict TZD response (8,10). We (13) and others (14) hypothesized that genetic variation in *PPARG* may account for lack of response to TZDs and examined whether the common P12A variant in *PPARG*, an accepted type 2 diabetes susceptibility variant (15–17), was associated with insulin sensitization in response to TZD treatment. Results from both studies found no evidence for association between this variant and TZD response.

Because TZDs are agonists for PPARG, we hypothesized that variants other than P12A might underlie differential response to TZD therapy among the participants of the TRIPOD study. Therefore, the goal of the present study was to screen the promoters, exons, exon-intron boundaries, and partial intronic sequence of *PPARG* to identify polymorphisms and test them for association with TZD response in the high-risk Hispanic women who participated in the TRIPOD study.

RESEARCH DESIGN AND METHODS

Subjects for this study were derived from the troglitazone treatment arm of the TRIPOD study (8,9). Among the 108 women randomized to the treatment arm who had intravenous glucose tolerance tests (IVGTTs) at randomization and 3 months later to assess drug response, the 93 who provided DNA samples

From the ¹Genetic Basis of Human Disease Division, Translational Genomics Research Institute, Phoenix, Arizona; the ²Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California; and the ³Department of Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California.

Address correspondence and reprint requests to Richard M. Watanabe, PhD, Keck School of Medicine, University of Southern California, Department of Preventive Medicine, 1540 Alcazar St., CHP-220, Los Angeles, CA 90089-9011. E-mail: rwatanab@usc.edu.

Received for publication 6 May 2005 and accepted in revised form 19 August 2005.

T.A.B. has been on an advisory panel for Takeda, Amylin, and Isis; has received consulting fees from Takeda, Novo Nordisk, Amylin, PPD, Isis, and Daiichi; and has received grant/research support from Takeda.

BAC, bacterial artificial chromosome; IVGTT, intravenous glucose tolerance test; MAF, minor allele frequency; PPARG, peroxisome proliferator-activated receptor- γ ; SNP, single nucleotide polymorphism; TRIPOD, Troglitazone in the Prevention of Diabetes; TZD, thiazolidinedione.

© 2005 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

TABLE 1
Subject demographics

	Responders		Nonresponders	
	Baseline	3 months	Baseline	3 months
Age (years)	35.7 (10.7)	—	37.7 (10.5)	—
BMI (kg/m ²)	29.0 (7.0)	29.0 (7.3)	30.0 (5.3)	30.0 (7.1)
Fasting glucose (mmol/l)	5.2 (0.7)	4.9 (0.7)	5.2 (0.9)	5.1 (0.7)*
Fasting insulin (pmol/l)	84 (60)	60 (24)	87 (66)	78 (42)*
S _i (×10 ³ min ⁻¹ per pmol/l)	2.09 (2.13)	3.80 (3.16)	2.41 (2.68)	2.54 (1.77)*
AIR (pmol/l × 10 min)	2,754 (2,818)	2,134 (2,178)	1,847 (1,586)	1,765 (1,742)
Disposition index	927 (847)	1375 (1,230)	853 (1,202)	591 (1,038)*

Data are median (interquartile range). Parameters are shown for baseline and after 3 months of treatment with troglitazone (400 mg/day). Nonresponse is defined as the lowest tertile in 3-month change in S_i. **P* < 0.05 between responders and nonresponders for the 3-month change from baseline. AIR, acute insulin response.

are the basis of the present report. All women were nondiabetic and provided written informed consent before being enrolled into the study. Nonresponders were defined as the women in the lowest tertile of 3-month change in S_i (median change $-0.1 \times 10^{-4} \text{ min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}$) from the IVGTT-based minimal model. Detailed information on these individuals has been previously published (8). Institutional review boards of the University of Southern California and Translational Genomics Research Institute approved this study.

IVGTT analysis. The methods used to assess metabolic parameters have been previously described in detail (8). Briefly, glucose and insulin profiles from the IVGTT were analyzed using the Bergman minimal model to derive whole-body S_i. The acute insulin response to glucose was computed as the incremental insulin area above basal for the first 10 min following glucose injection. The disposition index, a measure of β-cell compensation for insulin resistance, was calculated as the product of S_i and acute insulin response.

Identification of genetic variants. Primers were designed to amplify and sequence exons B-6 from the *PPARG* genomic sequence (NCBI accession no. AY157024), exons A1–A2 from bacterial artificial chromosome (BAC) clone 33519 (AC091492), the γ2 promoter from BAC clone 30G23 (AC090947), and the 3' flanking sequence from BAC clone 586C12 (AC027126). At the time of this study, there were seven single nucleotide polymorphisms (SNPs) within the *PPARG* genomic locus available on the HapMap website (<http://www.hapmap.org>, January 2004 build); those SNPs were also included in our investigation: rs1562041, rs880663, rs1151996, rs1175540, rs1152003, rs1152007, and rs709167. For these SNPs, primers were designed from sequences available in the SNP database (<http://www.ncbi.nlm.nih.gov/SNP>). Information on all sequencing primers is available upon request.

We screened ~40 kb of *PPARG* corresponding to all exons, exon-intron boundaries, ~2 kb of upstream regions flanking each of the three promoters, intron 2 and part of intron 5, as well as ~2 kb of 3' flanking region by direct sequencing using genomic DNA from all 93 women. Primers were designed to overlap by >100 bp, and average amplicon size was 600–800 bp. DNA was amplified in a final reaction volume of 10 μl using 60 ng genomic DNA, 10× standard PCR buffer containing 2.75 mmol/l MgCl₂ (Applied Biosystems, Foster City, CA), 200 μmol/l deoxyribonucleoside triphosphates, 0.24 μmol/l oligonucleotide primers, and 0.4 units AmpliTaq Gold (Applied Biosystems). PCR cycling conditions consisted of an initial denaturation at 96°C for 7 min, followed by 35 cycles of 96°C for 20 s, 57°C for 30 s, and 72°C for 45 s, ending with a final elongation step at 72°C for 5 min. Following amplification, 2.5 μl PCR product was treated at 37°C for 15 min/80°C for 15 min with 1 μl of ExoSAP-IT (USB, Cleveland, OH) to remove unconsumed deoxyribonucleoside triphosphates and oligonucleotide primers. Amplicons were bidirectionally sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and 35 cycles of 96°C for 10 s, 55°C for 10 s, and 60°C for 4 min. Sequences were resolved on the 3730xl DNA analyzer (Applied Biosystems).

Statistical analyses. The observed genotype frequency for each SNP was assessed for deviation from that expected under Hardy-Weinberg equilibrium. Association with troglitazone response was assessed by χ². Linkage disequilibrium and haplotype block structure was assessed using Haploview, version 3.0 (18). Haplotype blocks were determined using the method of Gabriel et al. (19), with low-frequency SNPs (minor allele frequency [MAF] <0.05) excluded from the analysis. We expected to observe an inflated number of significant *P* values (*P* < 0.05) given the level of linkage disequilibrium across *PPARG* and among the associated SNPs. Therefore, we performed permutation tests to assess the expected number of *P* values <0.05 given the observed level of linkage disequilibrium. We randomly permuted response status, repeated the association analysis for all markers, and tracked the number of *P* values <0.05 at each of 10,000 replicates.

For each haplotype block identified by Haploview, haplotype frequencies were estimated by expectation-maximization (20) and tested for association with troglitazone response using three classes of association tests: 1) the 2×*n* test, which uses a test of association for the 2×*n* table that arises for responders and nonresponders when there are *n* distinct haplotypes; 2) the best haplotype test, which was performed independently on responders and nonresponders to find the single haplotype that, when compared against all other haplotypes in a 2 × 2 table, gives the strongest evidence of association; and 3) the high versus low test, which combines in one group the haplotypes more frequent in the nonresponders and in another group the haplotypes more frequent in the responders and then calculates a 2 × 2 test of association for those data. A permutation test framework was used to assess statistical significance of the resulting test statistic for all tests.

SNP association with 3-month change in weight, fasting glucose, fasting insulin, and S_i was tested by linear regression analysis adjusting for age and 3-month change in BMI (except in the case of weight, which is a component of BMI), assuming dominant, recessive, and additive models. Phenotype values were statistically transformed to approximate univariate normality before analysis. Phenotype values are reported as unadjusted medians and interquartile ranges. *P* values are reported without correction for multiple testing.

RESULTS

Subject demographics. Characteristics of TRIPOD subjects have been described in detail (8). Table 1 shows baseline and 3-month post-treatment characteristics for 63 responders and 30 nonresponders who contributed data for the present report. The results did not differ signifi-

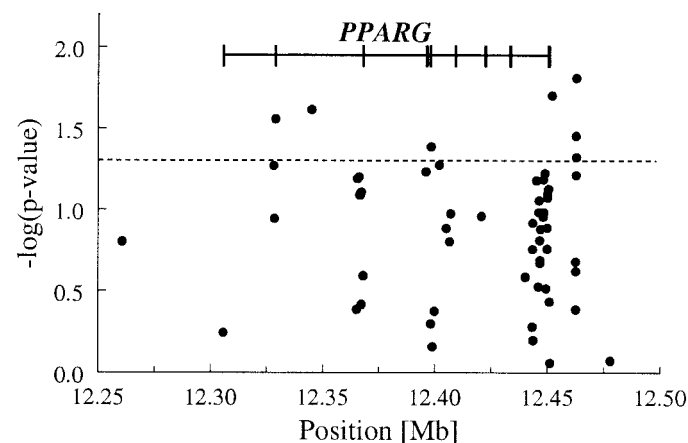


FIG. 1. Single marker association with response to troglitazone. The negative log of the *P* value for the χ² test of association is plotted according to physical distance. Horizontal dashed line denotes *P* value of 0.05. Two SNPs in close proximity gave identical *P* values, so only seven of the eight significant results are visible. The gene structure for *PPARG* is shown at the top with the A1 promoter on the left.

TABLE 2
SNP association with troglitazone response

SNP	Minor allele	MAF	OR (95% CI)	P value*
rs13073869	A	0.390	2.30 (1.09–4.87)	0.028
rs880663	C	0.400	2.36 (1.11–5.04)	0.024
rs4135263	C	0.291	2.37 (1.02–5.50)	0.041
rs1152003	G	0.410	2.19 (1.13–4.28)	0.020
rs6806708	T	0.378	0.46 (0.22–0.96)	0.035
rs13065455	A	0.391	2.04 (1.00–4.17)	0.047
rs13088205	G	0.436	2.36 (1.17–4.76)	0.016
rs13088214	C	0.391	2.04 (1.00–4.17)	0.047

P value is for χ^2 test of association with response to troglitazone for the minor allele.

cantly from the 15 women in the treatment arm for whom DNA samples were not available (data not shown).

Baseline characteristics did not differ significantly between the 63 responders and the 30 nonresponders, consistent with previously reported findings from the full 108 women in the TRIPOD treatment arm (8). As expected, responders had a greater increase in S_i at 3 months compared with nonresponders ($P < 0.0001$, Table 1). The change in S_i was associated with greater reductions in fasting glucose and insulin and a greater increase in disposition index compared with nonresponders (Table 1). Three responders (4.8%) and three nonresponders (10%) had study medication discontinued for a more than three-fold elevation of serum transaminase levels during the blinded trial.

SNP identification. We identified 133 *PPARG* variants, including 6 insertion-deletion polymorphisms (i.e., indels) and 127 SNPs. Of these SNPs, 54 were identified in the public SNP database, and all SNPs were genotyped as part of the HapMap project (as of 9 March 2004) and validated in this population. A total of 72 SNPs had MAF $< 5\%$ and were excluded from further analyses. The allelic distribution of one SNP (rs3105363) differed from that expected under Hardy-Weinberg equilibrium ($P = 0.009$) and therefore was excluded from further analyses.

Association with response to troglitazone. We first assessed individual SNP associations with response to troglitazone as a dichotomous trait (Fig. 1). Eight SNPs showed evidence for association ($P < 0.05$) and another 11 SNPs showed trends for association with response to troglitazone ($P < 0.08$, equivalent to 1.10 on the negative log scale). Table 2 summarizes the eight SNPs that were significantly associated with troglitazone response. SNPs rs13073869 and rs880663 were in complete linkage disequilibrium (Fig. 2) ($D' = 1.0$, $r^2 = 1.0$), and each showed the

same level of association with response ($P = 0.028$ and $P = 0.024$, respectively; values differ due to missing genotypes). These SNPs are located within the second intron of *PPARG*, between the A2 promoter and exon B. SNP rs1152003, located in the 3'-flanking region of *PPARG*, showed the strongest association with response ($P = 0.020$) and was not in linkage disequilibrium with any of the other associated SNPs or part of any haplotype block (Figs. 2 and 3). SNPs rs6806708, rs13065455, rs13088205, and rs13088214 were in strong linkage disequilibrium (Fig. 2) and were associated with response to troglitazone (Table 2). This cluster of SNPs is located downstream from rs1152003 in the 3'-flanking region of *PPARG*.

Permutation testing revealed that the probabilities of observing 8 P values < 0.05 and 19 total P values < 0.08 (8 P values < 0.05 plus the additional 11 P values ≥ 0.05 and < 0.08) were 0.0217 and 0.0071, respectively. Furthermore, the P value for association for each individual SNP was verified by permutation. There was only modest inflation of P values for all eight SNPs associated with response, and all remained significantly associated with troglitazone response.

We next examined the association between haplotype frequencies and response to troglitazone. Five haplotype blocks were identified (Fig. 3). Blocks 1–4 are situated within *PPARG* and block 5 is located in the adjacent 3'-flanking region of the gene. Block 1 starts within the first intron, spans the A2 promoter, and ends within intron 2. Block 2 is entirely situated within intron 2. Block 3 starts within intron 2; spans exons B, 1, and 2; and then ends within intron 5, which separates exons 2 and 3. Block 4 resides within intron 8, which separates exons 5 and 6.

Haplotype frequencies based on the first and second blocks showed evidence for association with response to troglitazone. There were five common haplotypes (frequency $> 1\%$ and two with frequency $> 30\%$) within block 1. The most frequent among the five haplotypes, T-A-G-T, had a frequency of 66.6% in nonresponders vs. 49.1% in responders, yielding an odds ratio (OR) for nonresponse of 2.22 ($P = 0.032$). There were three common haplotypes in block 2, and the most frequent, A-G-C-C-G (frequency 78.9%), was present with frequencies of 90.1 and 73.4% in nonresponders and responders, respectively (OR 4.18, $P = 0.012$). Within block 5 there were four common haplotypes, three with frequencies $> 20\%$. The most common among them (C-G-T-A-A-G-C) was marginally associated with troglitazone response (OR 0.51, $P = 0.049$).

Association with 3-month changes in phenotypes. SNPs were also tested for association with 3-month changes in fasting glucose, fasting insulin, body weight, and S_i assuming additive, dominant, and recessive genetic

r^2 \ D'	rs13073869	rs880663	rs4135263	rs1152003	rs6806708	rs13065455	rs13088205	rs13088214
rs13073869	1	1.0	0.84	0.09	0.35	0.35	0.35	0.35
rs880663	1	1	0.84	0.09	0.35	0.35	0.35	0.35
rs4135263	0.35	0.35	1	0.72	0.70	0.70	0.70	0.70
rs1152003	0	0	0.13	1	0.59	0.55	0.55	0.55
rs6806708	0.11	0.11	0.28	0.15	1	1	1	1
rs13065455	0.11	0.11	0.28	0.14	0.95	1	1	1
rs13088205	0.11	0.11	0.28	0.14	0.95	1	1	1
rs13088214	0.11	0.11	0.28	0.14	0.95	1	1	1

FIG. 2. Pair-wise linkage disequilibrium among SNPs associated with response.

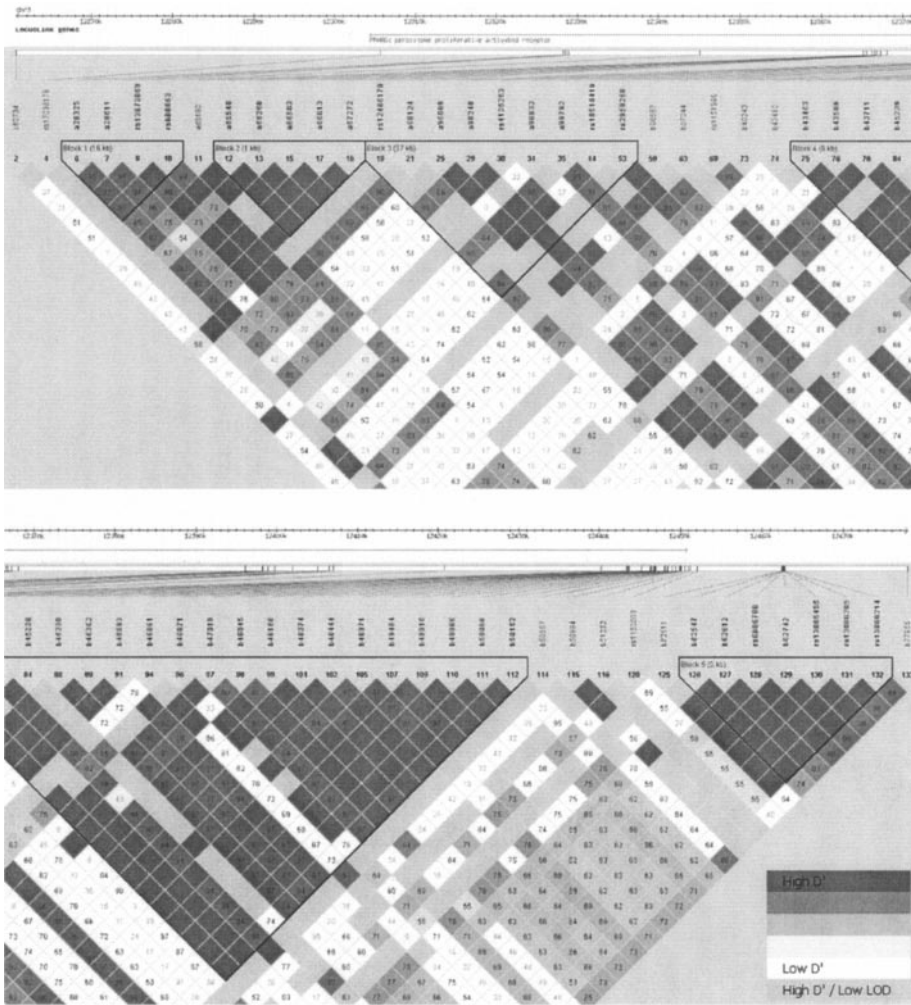


FIG. 3. *PPARG* pairwise linkage disequilibrium and haplotype block structure for the *PPARG* region. Haplotype blocks were estimated using the method of Gabriel et al. (19) (see RESEARCH DESIGN AND METHODS). The top and bottom panels split the region in half to facilitate viewing. Pairwise linkage disequilibrium among SNPs is represented by white (low D' , low logarithm of odds [LOD]), light gray (high D' , low LOD), and light to dark gray graded (high to low D' , high LOD) boxes.

models. None of the SNPs showing association with troglitazone response showed association with 3-month changes in fasting glucose or insulin (Table 3), except rs4135263, which showed modest association with change in fasting glucose under a recessive model ($P = 0.0447$). SNPs rs4135263 and rs10510419 both reside on haplotype block 3, are in perfect linkage disequilibrium ($D' = 1.0$, $r^2 = 1.0$) and showed significant association with change in weight under the recessive model (Table 3). Individuals homozygous for the minor allele gained less weight over the 3-month treatment period compared with other individuals. SNP rs1152003 also showed association with change in weight under both the additive and recessive models (Table 3). In contrast to the other SNPs, individuals homozygous for the minor allele for rs1152003 gained more weight than individuals with zero or one copy of the minor allele.

SNPs rs4135263 and rs10510419 both showed evidence for association with change in S_i under the recessive model (Fig. 4) ($P = 0.0473$ and $P = 0.030$, respectively; values differ due to missing genotype data). For both SNPs, individuals homozygous for the minor allele had much greater increases in S_i compared with other individuals (Table 3). SNP rs1152003 showed the strongest evidence for association with change in S_i under the recessive model (Table 3, $P = 0.019$). Individuals homozygous for the minor allele had a smaller change in S_i compared with other individuals (Table 3).

DISCUSSION

Few studies have attempted to elucidate mechanisms underlying individual differences in S_i changes in response to TZD therapy. The TRIPOD study was the first to show that baseline clinical and physiologic measures were not predictive of response to troglitazone (8). We (13) and others (14) also showed that the common Pro12Ala polymorphism in *PPARG* was not associated with response to TZD therapy. Recently, Kang et al. (21) showed association between variation in the adiponectin gene and response to rosiglitazone in Koreans with type 2 diabetes. In the current study, we extended our prior work on the genetics of response to troglitazone (13) by examining the remainder of *PPARG*. To our knowledge, no other study has examined the underlying genetics of TZD response using a carefully characterized and extensively phenotyped set of subjects. We identified five haplotype blocks in *PPARG* and surrounding regions, and association results based on response to troglitazone suggests that three of these may independently, or jointly, be involved in mediating response to troglitazone. This observed pattern of association was not altered by adjustment for minor baseline differences in age and S_i (data not shown). There was a single SNP that was located in the 3'-flanking region, rs1152003, which was not part of any haplotype block that also showed association with troglitazone response. Individual SNPs in blocks 1, 3, and 5, plus SNP rs1152003,

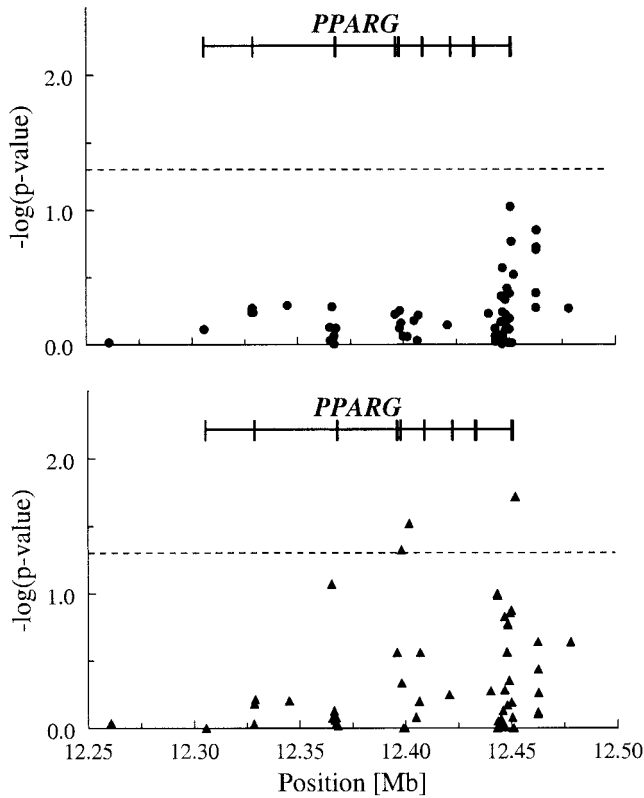


FIG. 4. Association with 3-month change in S_i . Individual SNPs were tested for association with 3-month changes in S_i assuming dominant (top panel) or recessive (bottom panel) models. The negative log of the P value for association is plotted according to physical distance. Horizontal dashed line denotes P value of 0.05.

showed association with 3-month changes in phenotypes, suggesting that these regions may be of importance in mediating troglitazone response.

Results based on haplotype frequencies were not completely consistent with the single SNP results. For example, block 2 consists of five SNPs of which four had

frequencies <5%. The single SNP with MAF >5% did not show evidence for association ($P = 0.331$). However, the most common haplotype in this block did show evidence for association. In contrast, rs4135263 showed evidence for association with troglitazone response, but haplotypes within this region only showed a trend for association. This pattern may be due in part to the fact that relative to the other blocks examined, block 3 is comprised of seven haplotypes, four of which have frequencies >10%, making it more difficult to detect associations.

When 3-month changes in S_i and related phenotypes were assessed as continuous traits, we did not observe any associations between the SNPs associated with dichotomous troglitazone response and 3-month continuous changes in fasting glucose or insulin (Table 3). However, we did observe associations with 3-month changes in both body weight and S_i . The fact that variants were associated with changes in these two phenotypes is consistent with the hypothesized mechanism of action of TZDs (22,23). Multiple clinical trials have demonstrated an increase in body weight with TZD therapy (3,24–26). Some of this increase is due to changes in fat accumulation and distribution (24,25), while some is accounted for by fluid retention due to TZD-induced effects on the vasculature (27) and sodium reabsorption (27,28). Thus, associations with changes in either or both phenotypes can be indicative of a TZD-induced response mechanism. In fact, we observed a modestly significant negative association between 3-month change in body weight and 3-month change in S_i (data not shown, $P = 0.042$), suggesting that changes in body weight and S_i over the 3-month treatment period may, in part, be due to a common response to troglitazone.

Two SNPs within block 3 and rs1152003 showed associations with both weight change and change in S_i (Table 3). The genotype-specific median values for weight change were clustered around zero for rs4135263 and rs10510419, suggesting that these associations with weight change may be spurious. Genotype-specific medians for change in S_i revealed larger differences, suggesting a clear and real association.

TABLE 3
Associations with 3-month changes (3 months – baseline) in phenotypes

SNP	Genotypes	Model-specific P value		
		Additive	Dominant	Recessive
rs4135263	CC CT TT			
<i>n</i>	4 34 41			
Δ Weight (kg)	0.0 (1.4) 0.0 (2.3) 0.0 (2.7)	0.2440	0.6250	0.0435
Δ Glucose (mmol/l)	-11.0 (16.5) -2.5 (6.0) -4.0 (7.0)	0.8945	0.2865	0.0447
Δ Insulin (pmol/l)	-4.0 (14.5) -3.0 (3.0) -3.0 (8.0)	0.8454	0.5632	0.4500
ΔS_i ($\times 10^3 \text{ min}^{-1}$ per pmol/l)	2.20 (2.38) 0.80 (1.22) 0.73 (1.49)	0.2161	0.5600	0.0473
rs10510419	GG GT TT			
<i>n</i>	45 39 5			
Δ Weight (kg)	0.0 (2.7) 0.2 (2.7) 0.0 (0.5)	0.3862	0.9710	0.0277
Δ Glucose (mmol/l)	-4.0 (7.0) -2.0 (6.0) -5.0 (15.0)	0.5637	0.1554	0.1280
Δ Insulin (pmol/l)	-3.0 (7.0) -3.0 (4.0) -2.0 (5.0)	0.9137	0.9160	0.6143
ΔS_i ($\times 10^3 \text{ min}^{-1}$ per pmol/l)	0.83 (1.56) 0.81 (1.56) 3.06 (2.21)	0.3348	0.8717	0.0303
rs1152003	CC CG GG			
<i>n</i>	32 45 13			
Δ Weight (kg)	-0.2 (3.0) -0.2 (2.7) 0.9 (1.8)	0.0465	0.2969	0.0169
Δ Glucose (mmol/l)	-3.5 (9.5) -3.0 (8.0) -2.0 (6.0)	0.8741	0.7989	0.5177
Δ Insulin (pmol/l)	-3.0 (7.5) -3.0 (5.0) -3.0 (8.0)	0.6878	0.6595	0.8621
ΔS_i ($\times 10^3 \text{ min}^{-1}$ per pmol/l)	1.31 (1.66) 0.83 (1.56) 0.43 (1.44)	0.0504	0.3008	0.0191

Genotype-specific values are given as unadjusted median (interquartile range). P values represent models with adjustment for age and BMI, except for Δ weight, which is adjusted for age only.

Haplotype block 3 spans a large segment of the coding region of *PPARG*, including exons B, 1, and 2. Although none of the associated SNPs were found within exonic sequence, it is possible that one or more of the variants may disrupt alternative splicing, resulting in a protein product with an impaired ability to interact with troglitazone or a critical cofactor of *PPARG* such as retinoid X receptor- α . Alternatively, these variants may affect regulatory elements located within this region or be in linkage disequilibrium with such elements. The associations with rs1152003 and haplotype block 5 suggest a possible role for elements within the 3'-flanking region of *PPARG*. There are no known regulatory elements in this region nor any known or predicted expressed sequence tags based on the most recent build of the human genome (HG17, May 2004). Thus, it is not yet clear what role, if any, variants in this region might play in mediating response to troglitazone.

Although this study is the first to report association of *PPARG* variants with response to troglitazone and troglitazone-induced changes in phenotypes, our sample size is relatively small: 63 responders and 30 nonresponders. Despite the relatively small sample size, we have sufficient statistical power (>80%) to detect robust allele frequency differences (>17%) for the single SNP analysis. This falls within the range of observed differences in MAF between responders and nonresponders in our sample (20–40%). Our power is weaker for the analysis of haplotype frequencies, due to the number of different haplotypes estimated for each block. However, given the number of SNPs tested, and the underlying linkage disequilibrium across the gene, results from permutation testing indicated that the number of significant associations we observed was greater than that expected under the null. Finally, for our phenotype association analyses, post hoc power calculations indicate 80% power to detect an effect size of 0.67 SE, which is approximately a change in S_i of $1.0 \times 10^{-4} \text{ min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}^{-1}$. Therefore, we believe the observed associations reflect true biologic effects and do not represent type I errors. However, it would be desirable to see our findings replicated in a separate population and with currently available TZDs.

In summary, we identified sequence variation in *PPARG* that may contribute to different insulin-sensitizing responses to troglitazone therapy in Hispanic women with previous gestational diabetes. Our results provide evidence that genetic variants within two haplotype blocks may help to determine the response. Whether the inability to respond to troglitazone therapy is due to an effect of these variants on troglitazone binding to *PPARG* or an impairment of the agonistic activity of troglitazone through disruption of interactions between *PPARG* and critical *PPARG* cofactors (e.g., retinoid X receptor- α) will require functional characterization studies. Furthermore, our results indicate that response to TZD therapy may not be accurately predicted by genotyping one or two variants within *PPARG*. Instead, prediction of response may require assessment of a cluster of genotypes, possibly across different genes. Nonetheless, our results provide the first evidence supporting the concept that variation within *PPARG* partly accounts for response to therapy with a TZD.

ACKNOWLEDGMENTS

This work was supported, in part, by funds awarded to R.M.W. from the Donald E. and Delia B. Baxter Foundation. R.M.W. is also supported by a grant from the Amer-

ican Diabetes Association. T.A.B. is supported by a Distinguished Clinical Scientist Award from the American Diabetes Association. This work was also supported by the University of Southern California General Clinical Research Center (M01-RR-00043).

The authors thank the women who volunteered to participate in the TRIPOD study.

REFERENCES

- Aronoff S, Rosenblatt S, Braithwaite S, Egan JW, Mathisen AL, Schneider RL, the Pioglitazone 001 Study Group: Pioglitazone hydrochloride monotherapy improves glycemic control in the treatment of patients with type 2 diabetes. *Diabetes Care* 23:1605–1611, 2000
- Lawrence JM, Reckless JPD: Pioglitazone. *Int J Clin Pract* 54:614–618, 2000
- Baba S: Pioglitazone: a review of Japanese clinical studies. *Curr Med Res Opin* 17:166–189, 2001
- Goldstein BJ: Differentiating members of the thiazolidinedione class: a focus on efficacy. *Diabetes Metab Res Rev* 18:S16–S22, 2002
- Nolan JJ, Ludvik B, Beerdsen P, Joyce M, Olefsky J: Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone. *N Engl J Med* 331:1188–1193, 1994
- Antonucci T, Whitcomb R, McLain R, Lockwood D: Impaired glucose tolerance is normalized by treatment with the thiazolidinedione troglitazone. *Diabetes Care* 20:188–193, 1997
- Dunaif A, Scott D, Finegood D, Quintana B, Whitcomb R: The insulin-sensitizing agent pioglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome. *J Clin Endocrinol Metab* 81:3299–3306, 1996
- Buchanan TA, Xiang AH, Peters RK, Kjos SL, Marroquin A, Goico J, Ochoa C, Tan S, Berkowitz K, Hodis HN, Azen SP: Preservation of pancreatic β -cell function and prevention of type 2 diabetes by pharmacological treatment of insulin resistance in high-risk hispanic women. *Diabetes* 51:2796–2803, 2002
- Azen SP, Peters RK, Berkowitz K, Kjos SL, Xiang A, Buchanan TA, the TRIPOD Study Group: TRIPOD (TRoglitazone In the Prevention Of Diabetes): a randomized, placebo-controlled trial of troglitazone in women with prior gestational diabetes mellitus. *Control Clin Trials* 19:217–231, 1998
- Buchanan TA, Xiang AH, Peters RK, Kjos SL, Berkowitz K, Marroquin A, Goico J, Ochoa C, Azen SP: Response of pancreatic β -cells to improved insulin sensitivity in women at high risk for type 2 diabetes. *Diabetes* 49:782–788, 2000
- Scherbaum WA, Göke B, the German Pioglitazone Study Group: Metabolic efficacy and safety of once-daily pioglitazone monotherapy in patients with type 2 diabetes: a double-blind, placebo-controlled study. *Horm Metab Res* 34:589–595, 2002
- Herz M, Johns D, Reviriego J, Grossman LD, Godin C, Duran S, Hawkins F, Lochnan H, Escobar-Jimenez F, Hardin PA, Konkoy CS, Tan MH, the GLAB Study Group: A randomized, double-blind, placebo-controlled, clinical trial of the effects of pioglitazone on glycemic control and dyslipidemia in oral antihyperglycemic medication-naïve patients with type 2 diabetes mellitus. *Clin Ther* 25:1074–1095, 2003
- Snitker S, Watanabe RM, Ani I, Xiang AH, Marroquin A, Ochoa C, Goico J, Shuldiner AR, Buchanan TA: Changes in insulin sensitivity in response to troglitazone do not differ between subjects with and without the common, functional Pro12Ala PPAR- γ 2 gene variant: results from the Troglitazone in Prevention of Diabetes (TRIPOD) study. *Diabetes Care* 27:1365–1368, 2004
- Blüher M, Lübben G, Paschke R: Analysis of the relationship between the Pro12Ala variant in the PPAR- γ 2 gene and the response rate to therapy with pioglitazone in patients with type 2 diabetes. *Diabetes Care* 26:825–831, 2003
- Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl M-C, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES: The common PPAR γ Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 26:76–80, 2000
- Barroso I, Luan J, Middelberg RPS, Harding AH, Franks PW, Jakes RW, Clayton D, Schafer AJ, O'Rahilly S, Wareham NJ: Candidate gene association study in type 2 diabetes indicates a role for genes involved in β -cell function as well as insulin action. *PLOS Biol* 1:41–55, 2003
- O'Rahilly S, Barroso I, Wareham NJ: Genetic factors in type 2 diabetes: the end of the beginning? *Science* 307:370–373, 2005
- Barrett JC, Fry B, Maller J, Daly MJ: Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21:263–265, 2005

19. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D: The structure of haplotype blocks in the human genome. *Science* 296:2225–2229, 2002
20. Clark AG: Inference of haplotypes from PCR-amplified samples of diploid populations. *Mol Biol Evol* 7:111–122, 1990
21. Kang ES, Park SY, Kim HJ, Ahn CW, Nam M, Cha BS, Lim SK, Kim KR, Lee HC: The influence of adiponectin gene polymorphism on the rosiglitazone response in patients with type 2 diabetes. *Diabetes Care* 28:1139–1144, 2005
22. Spiegelman BM: PPAR- γ : adipogenic regulator and thiazolidinedione receptor. *Diabetes* 47:507–514, 1998
23. Otto C, Lehrke M, Göke B: Novel insulin sensitizers: pharmacogenomic aspects. *Pharmacogenomics J* 3:99–116, 2003
24. Mori Y, Murakawa Y, Okada K, Horikoshi H, Yokoyama J, Tajima N, Ikeda Y: Effect of troglitazone on body fat distribution in type 2 diabetic patients. *Diabetes Care* 22:908–912, 1999
25. Kelly IE, Han TS, Walsh K, Lean MEJ: Effects of a thiazolidinedione compound on body fat and fat distribution of patients with type 2 diabetes. *Diabetes Care* 22:288–293, 1999
26. Akazawa S, Sun F, Ito M, Kawasaki E, Eguchi K: Efficacy of troglitazone on body fat distribution in type 2 diabetes. *Diabetes Care* 23:1067–1071, 2000
27. de Dios ST, Hannan KM, Dilley RJ, Hill MA, Little PJ: Troglitazone, but not rosiglitazone, inhibits Na/H exchange activity and proliferation of macrovascular endothelial cells. *J Diabetes Complications* 15:120–127, 2001
28. Chen L, Yang B, McNulty JA, Clifton LG, Binz JG, Grimes AM, Strum JC, Wallace Harrington W, Chen Z, Balon TW, Stimpson SA, Brown KK: GI262570, a peroxisome proliferator-activated receptor γ agonist, changes electrolytes and water reabsorption from the distal nephron in rats. *J Pharmacol Exp Therap* 312:718–725, 2005