

Variation in *PPARG* Is Associated With Longitudinal Change in Insulin Resistance in Mexican Americans at Risk for Type 2 Diabetes

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Context: Peroxisome proliferator-activated receptor gamma (*PPARG*) is a susceptibility locus for type 2 diabetes mellitus (T2DM). Although cross-sectional associations have been reported, primarily for Pro12Ala, few longitudinal studies in nondiabetic populations have been conducted.

Objective: This study aimed to examine whether and to what extent variation in *PPARG* is associated with longitudinal changes in anthropometric and metabolic traits in Mexican Americans at risk for T2DM.

Setting and Design: Subjects were participants of BetaGene, a family-based study of obesity, insulin resistance, and β -cell function, who completed a baseline and follow-up study visit ($n = 378$; mean followup, 4.6 ± 1.5 y). Phenotypes included body fat assessed by dual-energy x-ray absorptiometry; insulin sensitivity (S_i), acute insulin response, and β -cell function (disposition index; DI) were estimated from iv glucose tolerance tests with Minimal Model analysis. Eighteen tag single nucleotide polymorphisms (SNPs) capturing variation in a 156-kb region surrounding *PPARG* were tested for association with changes in longitudinal traits. *P*-values were Bonferroni-corrected for multiple testing.

Results: Six SNPs (rs2972164, rs11128598, rs17793951, rs1151996, rs1175541, rs3856806) were significantly associated with rate of change in S_i after adjustment for age, sex, and body fat percentage, but not with changes in adiposity. rs17793951 also had a significant effect on change in DI over time. Association between rs1175541 and change in S_i varied by changes in adiposity such that only carriers of the minor allele who reduced body fat over followup improved S_i . rs1306470 (captured Pro12Ala, $r^2 = 0.9$) was not associated with rates of change in any traits and its effects were not modified by changes in adiposity.

Conclusions: Variation in *PPARG*, but not Pro12Ala, contributes to declining S_i and concomitant deterioration in β -cell function in Mexican Americans at risk for T2DM. (*J Clin Endocrinol Metab* 100: 1187–1195, 2015)

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Abbreviations: AIR, acute insulin response; BMI, body mass index; CEU, European ancestral population; CHB, Asian ancestral population; DI, disposition index; GDM, gestational diabetes mellitus; IVGTT, iv glucose tolerance test; LD, linkage disequilibrium; MAF, minor allele frequency; MEX, Mexican ancestral population; MuTHER, Multiple Tissue Human Expression Resource; OGTT, oral glucose tolerance test; *PPARG*, Peroxisome proliferator-activated receptor gamma; S_i , insulin sensitivity; SNP, single nucleotide polymorphism; T2DM, type 2 diabetes mellitus; YRI, African ancestral population.

Peroxisome proliferator-activated Receptor gamma (*PPARG*) has long been recognized as a susceptibility locus for type 2 diabetes mellitus (T2DM). *PPARG* is expressed in adipocytes and encodes a nuclear transcription factor with a major role in adipocyte differentiation and function (1). In addition to associations with diabetes risk, variation in *PPARG* has also been shown to be associated with body mass index (BMI) (2–9), waist circumference (3, 4), waist-hip ratio (5), body fat distribution (4, 5, 8), fasting insulin (8–10), and insulin sensitivity (7, 9, 10) in nondiabetic individuals. However, other studies have failed to replicate these associations (11–16). Moreover, most of the studies that investigated these relationships were based on cross-sectional designs and/or focused primarily on the effects of Pro12Ala.

Among the studies that have examined longitudinal quantitative trait associations with *PPARG* (17–23), most examined Pro12Ala in relation to changes in body weight over time in the context of an intervention trial aimed at weight loss among obese and/or glucose-intolerant subjects. Two of these studies reported an association between the Ala allele and weight loss (19, 22), and one with weight gain (17), among individuals randomly assigned to placebo. In a population-based study of nondiabetic individuals in the United States, Fornage et al (20) observed opposite effects of the Ala allele for two racial groups; presence of an Ala allele was associated with a significant 15-year decline in adiposity among African Americans but an increase among whites. Significant decreases in fasting insulin and insulin resistance associated with Ala were only observed among African Americans (20). In a study of nondiabetic French individuals, Jaziri et al (23) reported that carriers of the Ala allele had significantly lower fasting insulin and insulin resistance after 6 years of followup compared with those with the Pro/Pro genotype. The inconsistent longitudinal findings reported to date within both homogenous and racial/ethnically diverse groups and scant data in the Mexican-American population underscore the need for investigation of the longitudinal effects of variation in *PPARG* in Mexican Americans at risk for T2DM.

The present study examined the effects of variation in *PPARG* on longitudinal changes in anthropometric and metabolic traits in a Mexican-American cohort enriched with individuals at high risk for T2DM. We also assessed whether the observed associations between the genetic variants and change in metabolic traits over time varied by changes in adiposity over time.

Materials and Methods

Subject recruitment

Participants of this study were from BetaGene, a family-based study of obesity, insulin resistance, and β -cell dysfunction in

Mexican Americans. Details regarding recruitment have been previously described (24). Briefly, probands qualified for participation if they 1) were of Mexican ancestry (both parents and at least 3/4 of grandparents Mexican or of Mexican descent, 2) had a confirmed diagnosis of gestational diabetes mellitus (GDM) or normal glucose levels (no GDM) during a pregnancy within the 5 years of study enrollment, and 3) had no evidence of β -cell autoimmunity by glutamic acid decarboxylase-65 testing. GDM and non-GDM probands were identified from the patient populations of Los Angeles County/University of Southern California Medical Center, Kaiser Permanente Southern California, and obstetrical/gynecological clinics at local southern California hospitals, and were frequency matched on age, BMI, and parity categories. Family members of GDM probands were recruited and included siblings, cousins, spouses, offspring, parents, and connecting aunts and uncles. Spouses and offspring of non-GDM probands were also invited to participate. Protocols for BetaGene were approved by the Institutional Review Boards of each institution, and all participants provided written informed consent prior to study enrollment.

Phenotyping for BetaGene was performed on two separate visits to the General Clinical Research Center. The first visit consisted of a physical examination, DNA collection, administration of food frequency and physical activity questionnaires, and a 2-hour 75-g oral glucose tolerance test (OGTT) with blood samples obtained before and at 30-minute intervals after glucose ingestion, as previously described (24). Fasting blood samples were also collected for lipid measurements. Participants with fasting glucose less than 126 mg/dL (< 7 mmol/L) were invited for a second visit, which consisted of a dual-energy x-ray absorptiometry scan for body composition and an insulin-modified iv glucose tolerance test (IVGTT) as previously described (24). All participants in the proband generation ($n = 1247$; 72% female; mean \pm SD age, 34.7 ± 8.2 y) underwent the full phenotyping protocol, whereas parents, uncles, aunts, spouses, and offspring had only a physical examination, fasting glucose measurement, and DNA collection.

The BetaGene follow-up study (BetaGene II) was designed to recall 400 participants from the proband generation of BetaGene for assessment of longitudinal changes in anthropometric and metabolic traits (25). Subjects with a fasting glucose level of at least 7.0 mmol/L were ineligible for further follow-up testing. Otherwise, both clinic visits described above were repeated on a subset of GDM probands, their siblings and cousins, and non-GDM probands ($n = 390$; 74% female; mean \pm SD age, 39.5 ± 8.4 y; mean \pm SD follow-up time, 4.6 ± 1.5 y). Due to follow-up study eligibility criteria, those who returned for followup had slightly lower median fasting and 2-hour glucose levels than those who did not participate ($P = .09$ and $P = .04$, respectively); no other significant differences between these two groups were observed (data not shown). We report results from 378 of the 390 BetaGene II participants, for whom complete genotype and phenotype data described below were available.

Assays

Plasma glucose was measured on an autoanalyzer using the glucose oxidase method (YSI Model 2300; Yellow Springs Instruments, Yellow Springs, OH). Insulin was measured by two-site immunoenzymometric assay (TOSOH Biosciences, San Francisco, CA) that has less than 0.1% cross reactivity with proinsulin and intermediate-split products.

Molecular analysis

Genotyping for BetaGene was performed on the Illumina OmniExpress platform. We selected 207 SNPs residing in *PPARG*, as well as eight SNPs in the regions 5-kb upstream and downstream of the gene for the current study. Genotype data were tested for non-Mendelian inheritance with MENDEL v.12.0 (26). We tested for deviation from Hardy-Weinberg equilibrium and estimated allele frequencies by maximum likelihood using SOLAR v.6.6.2 (27). Pair-wise linkage disequilibrium (LD) and haplotype block structure for the SNPs in *PPARG* were assessed using Haploview v.4.1 (28). Haplotype block structure was assessed using the method of Gabriel et al (29). After excluding SNPs for which the assay failed ($n = 3$), that failed ($n = 10$), were monomorphic ($n = 30$), or had minor allele frequencies (MAFs) less than 5% ($n = 75$), tag SNPs were selected from among the remaining 97 SNPs using TAGGER, with the default setting of pairwise $r^2 \geq 0.8$, as implemented in Haploview (28). We identified proxies for two common SNPs of interest that were not genotyped on the OmniExpress chip (rs10865710 and rs7649970) from the 1000 Genomes MXL panel (30).

For all BetaGene participants, we estimated proportions of admixture using a genome-wide set of 117 347 LD-pruned markers available on the OmniExpress array and the program ADMIXTURE v.1.21 (31). We included data for 591 individuals from the HapMap CEU, CHB, YRI, and MEX populations as proxies for European, Asian, African and Mexican ancestral populations, respectively. Assuming four subpopulations was appropriate for these data and yielded the lowest cross-validation error (data not shown). Proportions of admixture for BetaGene, compared with the HapMap CEU, CHB, YRI, and MEX populations are shown in Supplemental Figure 1.

Measures of insulin response to glucose

We calculated two measures of insulin response to glucose: the difference between the OGTT 30' and fasting plasma insulin ($30' \Delta \text{Insulin}$) and the incremental area under the insulin curve during the first 10 minutes of the IVGTT (acute insulin response; AIR). IVGTT glucose and insulin data were analyzed using the minimal model (MINMOD Millennium v.5.18) to derive insulin sensitivity (S_I) (32). β -cell function was measured by disposition index ($DI = S_I \times \text{AIR}$), a measure of β -cell compensation for insulin resistance (33).

Data analysis

Demographic characteristics for the BetaGene II cohort are described by median and corresponding 25th and 75th percentiles. Descriptive analyses were performed using SAS software v.9.2 (SAS Institute Inc, Cary, NC). Baseline quantitative traits with skewed distributions were statistically transformed to approximate univariate normality for cross-sectional analyses. The rates of change in AIR, S_I , and DI were computed as the difference between the log of the follow-up value and the log of the baseline value divided by total follow-up time; the rate of change in percent body fat was calculated as the difference between the follow-up value and baseline value, divided by total follow-up time. The resulting distributions for the rate of change in each trait were approximately normal. We tested each tag SNP for association with both baseline and the rate of change in anthropometric and metabolic traits using likelihood ratio tests within a variance components framework implemented in SOLAR (27), to account for familial correlation. An additive genetic model

was assumed for SNPs with MAF greater than 20%, and a dominant model otherwise. All models were adjusted for age, sex, and baseline BMI or body fat percentage (where appropriate). To determine whether results would be further affected by population substructure and/or changes in adiposity over time, we also examined models that were additionally adjusted for estimated proportions of admixture and/or change in body fat percentage.

P-values were Bonferroni corrected for 18 tag SNPs to account for multiple testing, although because of the modest level of correlation underlying many of the SNPs examined and the assumption of independence inherent in the applying a Bonferroni correction, corrected *P*-values are likely overly conservative. Age-, sex-, and percent body fat-adjusted geometric means and SEs, computed by the Delta Method (34), for S_I at baseline and mean followup, are presented by genotype for all tag SNPs. To determine whether the effect of the six SNPs associated with rate of change in S_I varied by changes in body fat percentage over time, we tested the interaction between each of the six SNPs and the rate of change in body fat percentage using a 1-df likelihood ratio test. *P*-values for the tests of interaction were Bonferroni-corrected for the six interaction tests performed.

We estimated a priori power to assess our ability to detect association between SNPs in *PPARG* and longitudinal change in S_I . For the lowest frequency SNP, which had a MAF = 11.0% (eg, Pro12Ala, or its proxy rs13064760) and was modeled under a dominant genetic model with genotype-specific sample sizes of 299 and 79 participants, respectively, we had 80% power to detect a difference of 0.08 in the mean rate of change in S_I ($\times 10^{-3}/\text{min per pmol/L per year}$) between the two groups, assuming a 100% coefficient of variation in each group, two-sided test of no difference and $\alpha = 0.0028$ (Bonferroni corrected for 18 SNPs). Under these assumptions, we had 80% power to detect a difference of 0.06 in the mean rate of change in S_I for a SNP with MAF = 20.0%.

Results

The sample was composed of 378 BetaGene II participants, of whom 74.3% were female, and the median (25th, 75th percentile) baseline age was 34.6 (29.4, 40.4) years. Median BMI and body fat percentage were 28.7 kg/m² and 36.1%, respectively. At baseline, median age, anthropometric, and metabolic profiles were generally similar for GDM probands and their family members, although GDM probands tended to be at the higher end and their cousins at the lower end of the range of values for each characteristic (Table 1). Non-GDM probands were similar to GDM probands in median age, BMI, and body fat percentage, by virtue of the fact that these two groups were initially frequency matched at baseline. However, non-GDM probands tended to have lower median OGTT-measured glucose and insulin, higher S_I and DI, and lower low-density lipoproteins and triglyceride levels than GDM probands (Table 1). Overall, study participants did not show statistically significant changes in any anthropometric traits between baseline and follow-up visits. However, deterioration in metabolic profile over time was evident; at

Table 1. Characteristics of the BetaGene Longitudinal Cohort (n = 378) at Baseline

Characteristic	GDM Probands (n = 79)	Siblings (n = 184)	Cousins (n = 80)	Non-GDM Probands (n = 35)
Female, n (%)	79 (100.0)	114 (62.0)	53 (66.3)	35 (100.0)
Age, y	35.2 (32.3, 39.9)	35.3 (29.0, 42.4)	32.5 (24.1, 38.7)	34.0 (31.4, 37.3)
Anthropometrics				
BMI, kg/m ²	30.1 (26.3, 32.8)	28.8 (26.2, 33.0)	27.0 (22.7, 30.5)	28.2 (24.3, 32.7)
Body fat percent	38.5 (36.3, 42.7)	34.3 (26.1, 40.4)	32.5 (23.8, 37.8)	38.4 (33.4, 40.5)
Trunk fat, kg	14.5 (12.7, 18.2)	12.4 (9.8, 17.5)	10.6 (7.7, 15.1)	11.9 (8.6, 17.2)
Waist circumference, cm	95.0 (89.5, 103.0)	94.0 (86.0, 103.0)	90.0 (78.0, 99.0)	88.0 (82.0, 96.5)
OGTT				
Fasting glucose, mmol/L	5.2 (4.8, 5.5)	5.1 (4.7, 5.4)	5.0 (4.7, 5.3)	4.7 (4.4, 4.9)
2-h glucose, mmol/L	8.0 (6.8, 9.6)	7.3 (6.1, 8.5)	6.4 (5.3, 7.7)	5.7 (4.9, 6.8)
30' Δ insulin, pmol/L	300 (162, 510)	339 (204, 558)	324 (201, 507)	306 (192, 564)
Fasting insulin, pmol/L	54 (36, 84)	42 (24, 72)	36 (18, 60)	30 (18, 48)
2-h insulin, pmol/L	468 (276, 798)	348 (228, 570)	273 (117, 402)	264 (180, 378)
IVGTT				
S ₁ , × 10 ⁻³ /min per pmol/L	2.46 (1.66, 3.72)	2.54 (1.69, 3.57)	3.15 (2.22, 4.69)	3.46 (2.24, 4.75)
AIR, pmol/L × 10 min	1682 (958, 3580)	2925 (1688, 4695)	2867 (1560, 4559)	3460 (2080, 4970)
DI	4966 (2746, 6934)	7528 (4628, 11 341)	7903 (5664, 12 056)	11, 379 (7297, 14 228)
Lipids				
Cholesterol, mg/dL	168 (152, 196)	173 (156, 199)	165 (149, 183)	151 (141, 172)
HDL, mg/dL	46 (40, 52)	45 (38, 50)	49 (41, 57)	50 (42, 61)
LDL, mg/dL	100 (79, 121)	106 (87, 127)	97 (77, 111)	85 (71, 102)
Triglycerides, mg/dL	100 (76, 158)	110 (68, 161)	78 (46, 114)	67 (41, 101)

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Unless otherwise stated, values are median (25th, 75th percentile).

followup, participants had significantly higher median OGTT-measured 2-hour glucose and insulin, lower S₁ and DI, and higher triglyceride levels than they did at baseline (data not shown).

Estimated pairwise LD and haplotype block structure for the 97 SNPs included in this analysis are shown in Supplemental Figure 2. Among these 97 SNPs, we identified 18 tag SNPs (Table 2 and Supplemental Figure 3). Tag SNP MAFs ranged from 11.0–43.3%. C34G (Pro12Ala; rs1801282) was tagged by rs13064760 ($r^2 = 0.9$). C1431T (rs3856806) was captured as a tag SNP. C-681G (rs10865710) and C-689T (rs7649970) were tagged by rs11128598 ($r^2 = 1.0$) and rs17036328 ($r^2 = 1.0$), respectively.

After adjustment for age and sex and correction for multiple testing, none of the 18 tag SNPs were significantly associated with any of the anthropometric or metabolic traits at baseline (all corrected $P \geq .20$). Moreover, none were associated with any cross-sectional traits at followup (all corrected $P \geq .10$). Cross-sectional associations with metabolic traits remained similar after further adjustment for BMI or body fat percentage. None of the SNPs were significantly associated with the rate of change in anthropometric traits; rs2972164 was only marginally associated with the rate of change in BMI (corrected $P = .08$). However, six SNPs: rs2972164, rs11128598, rs17793951, rs1151996, rs1175541 and rs3856806, were significantly associated with the rate of change in S₁ in models adjusted

for age, sex, and baseline body fat percentage (corrected $P = .006, .037, .012, .004, .016, \text{ and } .037$ respectively). Age-, sex- and percent body fat–adjusted mean values of S₁ at baseline and follow-up are shown by genotype for each of the 18 tag SNPs in Table 3. In general, the minor alleles of rs2972164, rs17793951, rs1151996, and rs1175541 were associated with slower rates of decline in S₁, whereas those for rs11128598 and rs3856806 were associated with faster rates of decline. Only rs17793951 was associated with the rate of change in DI (corrected $P = .05$). Adjusted mean values of S₁, DI, and body fat percentage at baseline and followup are shown for rs17793951 in Figure 1. Individuals carrying a G allele at this locus manifested little change in S₁ or DI over time, whereas those homozygous for the A allele decreased S₁ with concomitant decreases in DI, although there were no differences in the rate of change in body fat percentage by genotype (Figure 1). Results were similar after adjusting models for baseline BMI instead of body fat percentage, or further adjusting models for changes in adiposity (eg, BMI or body fat percentage) over time. Additional adjustment for population substructure did not alter these findings. Analysis of female participants only, most of the cohort, yielded similar estimates although some of these results became nonsignificant after multiple testing correction due to reduced sample size.

Among the six SNPs associated with rate of change in S₁, only the association with rs1175541 varied signifi-

Table 2. Characteristics of 18 Tag SNPs in *PPARG*^a

SNP	Position	Location	Major Minor Allele	MAF
rs13076933	12327431	upstream	T G	13.5%
rs2972164	12334416	intron	C T	30.6%
rs11128598 ^b	12353326	intron	T C	41.3%
rs17036281	12354411	intron	T G	28.2%
rs13064760 ^c	12369401	intron	C T	11.0%
rs17793951	12370737	intron	A G	16.8%
rs17036328 ^d	12390484	intron	T C	12.7%
rs4135268	12437237	intron	C G	11.0%
rs4135275	12443844	intron	A G	23.4%
rs1151996	12445807	intron	T G	20.4%
rs1151998	12446492	intron	T C	43.3%
rs1175541	12465488	intron	C A	18.9%
rs13099634	12468463	intron	C T	33.8%
rs1152001	12471862	intron	T C	17.7%
rs1152002	12471871	intron	G A	29.4%
rs3856806 ^e	12475557	exon 6	C T	12.0%
rs4135300	12475994	3' UTR	C T	11.4%
rs9833097	12478817	downstream	G A	28.5%

^a Genome build GRCh37/hg19.

^b Tag for C-681G (rs10865710); pairwise $r^2 = 1.0$, based on 1000 Genomes MXL data (35).

^c Tag for Pro12Ala (rs1801282); pairwise $r^2 = 0.9$, based on BetaGene data.

^d Tag for C-689T (rs7649970); pairwise $r^2 = 1.0$, based on 1000 Genomes MXL data (35).

^e SNP C1431T.

cantly with rate of change in body fat percentage (corrected interaction $P = .013$). Adjusted mean values by rs1175541 genotypes, stratified by rate of change in body fat percentage > 0 (increased body fat over time) and ≤ 0 (no change or reduced body fat over time) are shown in Figure 2. Overall, 233 participants (62% of cohort) increased body fat during followup. Among these individuals, S_1 decreased over time regardless of genotype (corrected $P = .10$). However, among individuals whose body fat percentage remained the same or decreased over time, S_1 increased for those carrying the minor allele, but continued on a decreasing trend in those homozygous for the major allele (corrected $P = .029$).

Discussion

In this Mexican-American cohort enriched with individuals at risk for T2DM, we observed association between variation in *PPARG* and the rate of decline in S_1 over time, independent of cross-sectional or longitudinal changes in adiposity. We also found the effect of one SNP, rs1175541, on the rate of change in S_1 to vary by longitudinal changes in body fat percentage. To our knowledge, this is the first study to demonstrate differential effects of

variation in *PPARG* on insulin sensitivity by changes in adiposity over time.

In a single-arm intervention study of 95 overweight and obese Japanese women assigned to 14 weeks of caloric restriction (~ 1200 kcal/d), Matsuo et al (21) reported significant decreases in several anthropometric and metabolic traits such as body weight, body fat percentage, triglycerides, and fasting glucose. Matsuo et al also found that changes in adiposity over the course of the trial varied significantly by *PPARG* genotypes. Specifically, study participants carrying minor alleles for any one of six SNPs (rs2959272, rs1386835, rs709158, rs1175540, rs1175544, rs1797912), all of which are located in a large intron of *PPARG*, experienced significantly greater weight reduction than those homozygous for the major allele. The largest and most significant weight-loss association was observed with rs1175544, which was also associated with decreases in waist circumference and fat mass. In BetaGene, rs1175544 is in strong LD with rs1175541 ($r^2 = 0.9$), whereas its MAF is only half of that observed in the Japanese study (19 vs 38%). Although rs1175541 was not significantly associated with changes in any anthropometric traits in our study, it was associated with the rate of change in S_1 . Furthermore, it significantly interacted with change in body fat over time, such that improvement in S_1 due to reduction in body fat percentage was only apparent among those carrying the minor allele. Taken together, these data suggest that rs1175541, and/or other intronic variants in LD with it, may have an important regulatory role in body composition and concomitant changes in insulin sensitivity over time.

Two variants previously shown to be associated with BMI and/or body composition in Caucasian samples, C-681G (rs10865710; tagged by rs11128598) and C1431T (rs3856806) (8, 11, 39), were not significantly associated with baseline or change in adiposity in the BetaGene longitudinal cohort. However, these variants were associated with change in S_1 over time. To date, studies of these variants have been conducted in other European, African American, Indian, and Asian populations (6, 9, 23, 35–39), although associations have not been replicated in nonwhite populations. Although the MAF for C1431T in BetaGene participants was similar to that typically observed among white individuals (12 vs 12–13%), the MAF for C-681G (or its proxy) was strikingly different (41 vs 25–26%). Further, these reports were based on cross-sectional analyses, making direct comparisons of SNP associations with longitudinal traits difficult. Interestingly, Wei et al (6) found that white individuals carrying the T allele of C1431T or the G allele of C-681G had a higher BMI than those homozygous for the C allele of either SNP. A few studies in nonwhite populations,

Table 3. Mean^a S_I at Baseline and at Mean Follow-up Time (4.6 y) by Genotype, According to Additive or Dominant Genetic Models as Appropriate, for 18 tag SNPs in *PPARG*

SNP	$S_I \times 10^{-3}/\text{min per pmol/L}$				P-Value ^c
	Baseline	SE ^b	Mean Followup (4.6 y)	SE ^b	
rs13076933					.185
TT	2.73	0.08	2.08	0.13	
TG/GG	2.56	0.13	2.41	0.17	
rs2972164					.006
CC	2.76	0.10	2.00	0.16	
CT	2.65	0.10	2.26	0.13	
TT	2.39	0.18	2.59	0.24	
rs11128598					.037
TT	2.67	0.13	2.38	0.13	
TC	2.62	0.09	2.12	0.14	
CC	2.92	0.18	1.86	0.26	
rs17036281					1.0
TT	2.63	0.10	2.20	0.11	
TG	2.63	0.10	2.14	0.14	
GG	3.33	0.23	2.01	0.50	
rs13064760					1.0
CC	2.69	0.08	2.22	0.10	
CT/TT	2.65	0.12	1.95	0.22	
rs17793951					.012
AA	2.76	0.09	2.04	0.13	
AG/GG	2.52	0.11	2.41	0.15	
rs17036328					1.0
TT	2.68	0.08	2.23	0.11	
TC/CC	2.67	0.12	1.96	0.19	
rs4135268					1.0
CC	2.69	0.08	2.19	0.11	
CG/GG	2.66	0.18	2.06	0.19	
rs4135275					1.0
AA	2.64	0.08	2.09	0.13	
AG	2.75	0.12	2.34	0.15	
GG	2.70	0.30	1.89	0.36	
rs1151996					.004
TT	2.83	0.09	2.04	0.14	
TG	2.49	0.11	2.35	0.15	
GG	2.34	0.20	2.39	0.26	
rs1151998					1.0
TT	2.60	0.11	2.23	0.15	
TC	2.68	0.10	2.16	0.15	
CC	2.83	0.17	2.06	0.20	
rs1175541					.016
CC	2.83	0.09	2.08	0.14	
CA/AA	2.45	0.10	2.30	0.13	
rs13099634					1.0
CC	2.55	0.10	2.14	0.11	
CT	2.74	0.10	2.22	0.17	
TT	3.04	0.22	2.05	0.29	
rs1152001					.118
TT	2.68	0.08	2.30	0.11	
TC/CC	2.68	0.11	1.89	0.17	
rs1152002					.100
GG	2.91	0.10	2.11	0.16	
GA	2.46	0.09	2.17	0.12	
AA	2.69	0.25	2.43	0.23	
rs3856806					.037
CC	2.66	0.08	2.27	0.11	
CT/TT	2.75	0.14	1.82	0.21	

(Continued)

Table 3. Continued

SNP	$S_I \times 10^{-3}/\text{min per pmol/L}$				P-Value ^c
	Baseline	SE ^b	Mean Followup (4.6 y)	SE ^b	
rs4135300					1.0
CC	2.71	0.08	2.20	0.11	
CT/TT	2.59	0.18	2.01	0.19	
rs9833097					1.0
GG	2.68	0.10	2.23	0.12	
GA	2.63	0.10	2.12	0.14	
AA	2.97	0.21	1.88	0.38	

^a Values shown are geometric means, adjusted for age, sex, and baseline body fat percentage.

^b SEs computed by the δ Method (38).

^c P-value for the association between the rate of change in S_I and SNP, under an additive or dominant genetic model as appropriate, adjusted for age, sex, and baseline body fat percentage. P-values are Bonferroni-corrected for the 18 tag SNPs tested.

whereas finding no significant association between these alleles and obesity, have observed their association with markers of insulin resistance (9), proinflammatory adipokines (39), or risk for impaired glucose tolerance and T2DM (37). In our study, the 1431T and -681G alleles (or the minor allele of the proxy SNP) were both associated with faster decline in S_I , which suggests a consistent allelic effect on anthropometric and metabolic traits in different racial/ethnic groups. Interestingly, C1431T (rs3856806) has been reported as a cis eQTL associated with significant increases *PPARG* expression in adipose tissue of 166 female subjects from the Multiple Tissue Human Expression Resource (MuTHER) study (40). Adipose-specific cis eQTL effects for this SNP were also replicated at a nominal level in an additional 856 female subjects from MuTHER (41). Additional experimental studies suggest that C1431T resides in a region that is highly conserved, hypersensitive to DNase, and a putative transcription factor binding site (42, 43). Taken together, the evidence suggests that this synonymous variant may also have an important *PPARG* regulatory function.

Similarly, Pro12Ala (rs1801282; tagged by rs13064760) and C-689T (rs7649970; tagged by rs17036328) were not associated with baseline levels of or rates of change in anthropometric or metabolic traits. In a population-based longitudinal study of nondiabetic French individuals, Jaziri et al (23) observed significant effects of Pro12Ala on 6-year change in fasting insulin and homeostasis model of assessment–insulin resistance, a surrogate measure of insulin resistance. Although associations between Pro12Ala and baseline or changes in anthropometric traits were not reported, the average BMI of these individuals was lower and in the normal range ($\sim 24 \text{ kg/m}^2$) (23), whereas that of our study participants was much higher ($\sim 29 \text{ kg/m}^2$).

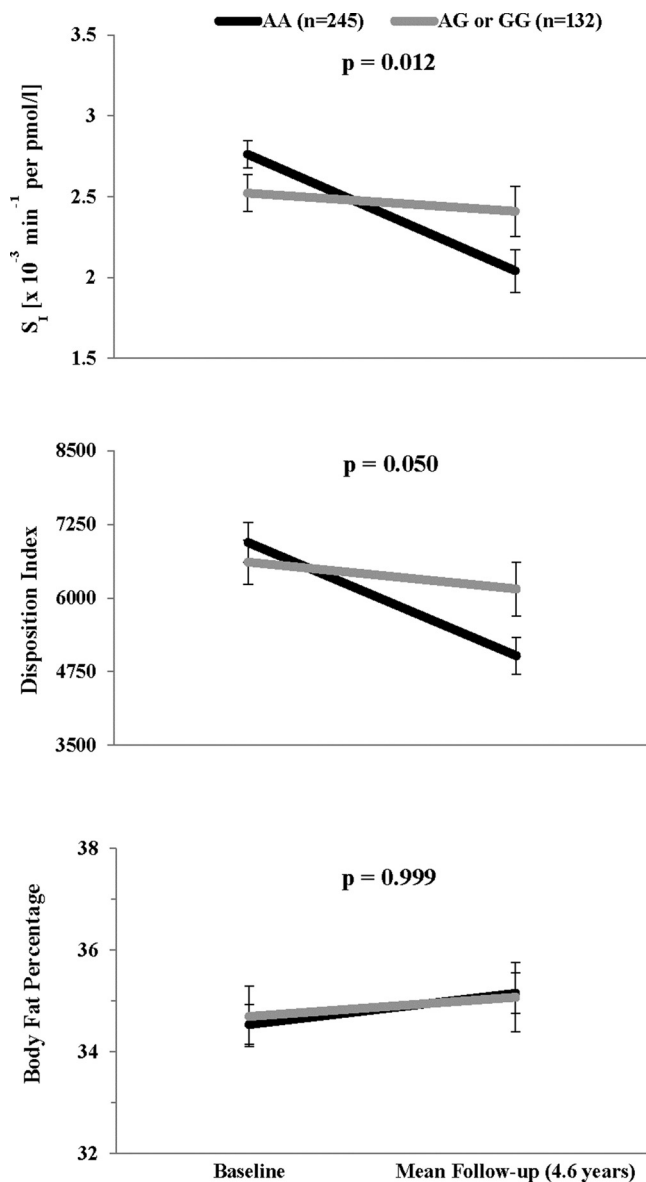


Figure 1. Association between rs17793951 and changes in S_1 , DI, and body fat percentage over followup. Black lines indicate genotype AA; gray lines indicate genotypes AG or GG. Values shown for S_1 and DI at baseline and at mean follow-up time (4.6 y) are geometric means with SEs computed by delta method, adjusted for age, sex, and body fat percentage. Values shown for body fat percentage are arithmetic means and SEs, adjusted for age and sex. *P*-values are Bonferroni corrected for 18 tag SNPs.

Conversely, in a study of nondiabetic African American and white individuals in the United States, Fornage et al (20) reported Pro12Ala associations with 15-year changes in anthropometric traits in both racial groups, but significant effects on change in homeostasis model of assessment were observed only among African Americans. Interestingly, this association was slightly attenuated and became nonsignificant after adjusting for baseline BMI (20), despite the fact that Pro12Ala was not significantly associated with BMI among African Americans in this study.

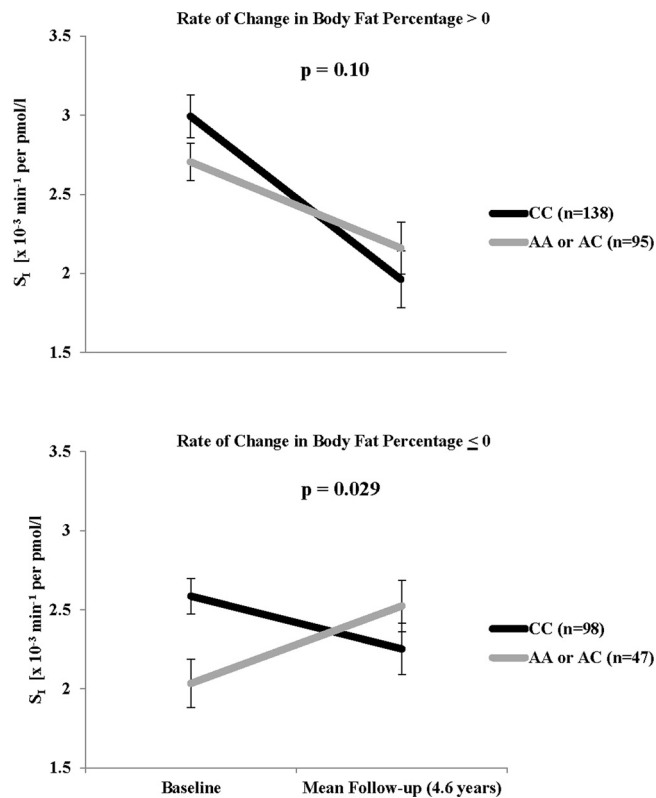


Figure 2. Change in S_1 over followup for rs117541, stratified by rate of change in body fat percentage. Black lines indicate genotype CC; gray lines indicate genotypes AA or AC. Values shown at baseline and at mean follow-up time (4.6 y) are geometric means and SEs, adjusted for age and sex. *P*-values are Bonferroni corrected for six interaction tests.

Few studies have examined the effects of variation in *PPARG* specifically in Mexican or Mexican-American populations (3, 24, 44, 45); all were cross sectional and focused exclusively on associations with Pro12Ala. One study reported significant associations between Pro12Ala and measures of adiposity (3), whereas the others failed to replicate this finding. We previously reported no main effect of Pro12Ala on cross-sectional anthropometric or metabolic traits in the full BetaGene cohort (24). Thus it is not surprising that we observed no such associations in the BetaGene II cohort. This report is the first to examine longitudinal changes in adiposity, insulin sensitivity, and β -cell function associated with variation in *PPARG* in Mexican Americans at risk for T2DM.

Strengths of our study also include our family-based design and ascertainment of study participants with parents and at least 3/4 grandparents of Mexican ancestry, both of which partially protect from population stratification in the study design, as well as our ability to statistically control for admixture with genome-wide data. Moreover, our longitudinal design and detailed phenotyping with dual-energy x-ray absorptiometry and IVGTTs for estimation of body fat percentage and direct assessment of insulin sensitivity and β -cell function, re-

spectively, are advantageous for studying physiological changes over time that precede T2DM. However, our study is not without limitations. Despite the unique array of genotype and longitudinal trait data available in BetaGene participants, the sample size of the subset with follow-up phenotyping is relatively modest. Given the multiple testing penalty applied, it is possible that we were unable to detect variation in *PPARG* associated with very small changes in body fat or S_I , particularly for low-frequency SNPs with small effects. However, we were well-powered to detect differences in the mean rate of change in these traits for the SNPs identified in this report. Finally, our study design did not afford us the opportunity to assess T2DM development among all study participants; further investigation of the direct contribution of these variants to T2DM risk in Mexican Americans is warranted.

In conclusion, we did not observe associations between any variants in *PPARG* and baseline levels of or changes in measures of adiposity. We did find that variation in *PPARG*, but not Pro12Ala, contributes to declining S_I and concomitant deterioration in β -cell function, after accounting for adiposity. The beneficial metabolic effects of weight loss or reductions in body fat may also depend on variation in *PPARG*. These data may have important implications for the treatment of obesity and prevention of T2DM in Mexican Americans.

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