

Evidence of Interaction Between *PPARG2* and *HNF4A* Contributing to Variation in Insulin Sensitivity in Mexican Americans

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OBJECTIVE—We hypothesized that interaction between *PPARG2* Pro12Ala and variants in the promoter region of *HNF4A* are associated with type 2 diabetes-related quantitative traits in Mexican-American families of a proband with previous gestational diabetes.

RESEARCH DESIGN AND METHODS—The BetaGene project genotyped *PPARG2* Pro12Ala and nine *HNF4A* single nucleotide polymorphisms (SNPs) in 473 individuals in 89 families. Members of the proband generation had fasting glucose <126 mg/dl and were phenotyped by oral and intravenous glucose tolerance tests.

RESULTS—Neither *PPARG2* Pro12Ala nor any of the nine *HNF4A* SNPs were independently associated with type 2 diabetes-related quantitative traits. However, the interaction between *PPARG2* Pro12Ala and *HNF4A* rs2144908 was significantly associated with both insulin sensitivity (S_I) (Bonferroni $P = 0.0006$) and 2-h insulin (Bonferroni $P = 0.039$). Subjects with at least one *PPARG2* Ala allele and homozygous for the *HNF4A* rs2144908 A allele had 40% higher S_I compared with individuals with at least one G allele. S_I did not vary by rs2144908 genotype among

PPARG2 Pro/Pro. The interaction result for S_I was replicated by the Insulin Resistance Atherosclerosis Family Study ($P = 0.018$) in their San Antonio sample ($n = 484$) where subjects with at least one *PPARG2* Ala allele and homozygous for the *HNF4A* rs2144908 A allele had a 29% higher S_I compared with individuals with at least one G allele. However, the interaction was not replicated in their San Luis Valley sample ($n = 496$; $P = 0.401$).

CONCLUSIONS—Together, these results suggest that variation in *PPARG2* and *HNF4A* may interact to regulate insulin sensitivity in Mexican Americans at risk for type 2 diabetes. *Diabetes* 57:1048–1056, 2008

Mexican-American women with previous gestational diabetes mellitus (GDM) exhibit significant β -cell dysfunction and are at high risk for developing type 2 diabetes (1–3). Moreover, their risk for future type 2 diabetes can be significantly reduced by improving insulin sensitivity and reducing insulin secretory demands (4). This propensity for β -cell failure in the face of chronic insulin resistance led us to design a family-based study, the BetaGene Study, to identify possible genetic determinants underlying this β -cell defect. In BetaGene, we performed detailed phenotyping of Mexican-American probands with recent GDM and their family members to obtain quantitative estimates of insulin sensitivity (S_I), acute insulin response (AIR), and β -cell compensation (disposition index), which are traits that we have shown to be heritable in Mexican-American families (5,6).

PPARG2 is a lipid-activated transcription factor that has a key role in the expression of genes involved in adipocyte differentiation and function, as well as regulation of genes in several other tissues (7,8). The common Pro12Ala polymorphism in *PPARG2* has been reported to be associated with type 2 diabetes (9–12) and with changes in plasma insulin levels (9,12,13) and insulin sensitivity (9) and thus is an accepted diabetes susceptibility variant. (*HNF4A*) is a transcription factor that regulates a vast network of genes involved in insulin secretion and glucose regulation (14). Mutations in the coding region of *HNF4A* have been shown to confer susceptibility to maturity-onset diabetes of the young (15). Common variants in the promoter region of *HNF4A* were initially shown to be associated with type 2 diabetes in Finns (16) and Ashkenazi Jews (17); some subsequent studies have replicated the association (18,19), although others have not (20,21). In Finns, variation in the P2-promoter region was also associated with measures of insulin secretion and β -cell

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Received for publication 22 June 2007 and accepted in revised form 19 December 2007.

Published ahead of print at <http://diabetes.diabetesjournals.org> on 25 January 2008. DOI: 10.2337/db07-0848.

M.H.B. and T.E.F. made equal intellectual contributions to and share first authorship of this work.

Additional information for this article can be found in an online appendix at <http://dx.doi.org/10.2337/db07-0848>.

AIR, acute insulin response; GDM, gestational diabetes mellitus; IRASFS, Insulin Resistance Atherosclerosis Family Study; IVGTT, intravenous glucose tolerance test; MTTP, microsomal triglyceride transfer protein; OGTT, oral glucose tolerance test; SNP, single nucleotide polymorphism.

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function in nondiabetic offspring of patients with type 2 diabetes (16).

Given the evidence that variants of both *PPARG2* and *HNF4A* play a role in diabetes risk and variation in diabetes-related traits, we tested whether these variants are associated with type 2 diabetes-related quantitative traits in BetaGene. We then tested our positive results in two similarly phenotyped Mexican-American samples from the Insulin Resistance Atherosclerosis Family Study (IRASFS), one from San Antonio, Texas, and the other from the San Luis Valley in Colorado (22).

RESEARCH DESIGN AND METHODS

Subject recruitment. Subject recruitment for BetaGene is ongoing and is briefly described in the online appendix (available at <http://dx.doi.org/10.2337/db07-0848>). For the purposes of this report, we describe only those clinical protocols and assays relevant to the results presented herein. Participation in BetaGene is restricted to Mexican Americans with fasting glucose <126 mg/dl (7 mmol/l) from families of a proband with GDM diagnosed within the previous 5 years who have available for study either two nondiabetic siblings and three nondiabetic first cousins from a single nuclear family or at least five siblings. The probands, siblings, and cousins have extensive phenotyping for diabetes-related traits (below) and form the basis for the primary analysis of this report.

IRASFS subject characteristics and ascertainment have been previously described (22). Briefly, probands were identified from the IRAS cohort study (23). IRASFS probands and their family members were recruited without regard to diabetes or glucose tolerance status. Mexican-American participants in IRASFS were from San Antonio, Texas, or the San Luis Valley in Colorado.

All protocols for BetaGene and the IRASFS were approved by the Institutional Review Boards of participating institutions, and all participants provided written informed consent before participation.

Clinical protocols. Phenotyping for BetaGene is performed on two separate visits to the General Clinical Research Center. Visit 1 consists of a physical examination, DNA collection, a 75-g oral glucose tolerance test (OGTT), and fasting blood for lipid measurements. Visit 2 consists of a dual-energy X-ray absorptiometry scan for determination of body fat and an insulin-modified intravenous glucose tolerance test (IVGTT) performed as previously described (4). Proband, their siblings, and their cousins undergo the full phenotyping protocol, whereas parents, uncles, aunts, spouses, and offspring had only a physical exam, fasting glucose measurement, and DNA collection.

Details of the IRASFS clinical exams and phenotype measurements have been published (22). Of particular relevance for this report, the IVGTTs for IRASFS were performed using the same protocol as was used in the BetaGene study, but no OGTT was performed in IRASFS.

Assays. In both BetaGene and IRASFS, plasma glucose was measured on an autoanalyzer using the glucose oxidase method (YSI Model 2300; Yellow Springs Instruments, Yellow Springs, OH). In BetaGene, insulin was measured by two-site immunoenzymometric assay that has <0.1% cross-reactivity with proinsulin and intermediate split products; whereas in IRASFS, insulin was measured by radioimmunoassay with dextran-charcoal separation (24).

Molecular analysis. In BetaGene, we attempted to genotype the *PPARG2* Pro12Ala variant (rs1801282) and 10 *HNF4A* single nucleotide polymorphisms (SNPs) showing evidence for association with type 2 diabetes in Finns (16): rs4810424, rs1884613, rs1884614, rs2144908, rs6031551, rs6031552, rs2425637, rs2425640, rs3212183, and rs1885088. The assay for rs3212183 failed, but genotype data for the remaining nine SNPs were obtained. SNP genotyping was performed using the Applied Biosystems TaqMan system (25). Genotyping assays were either selected through the Assays on Demand database (Applied Biosystems; <http://myscience.appliedbiosystems.com/navigation/mysciapplications.jsp>) or custom designed using the Assays by Design service (Applied Biosystems). Based on 22 blinded duplicate samples, the discrepancy rate for genotyping was 0%, and the overall genotype success rate was >97.6%.

IRASFS genotyped the *PPARG2* Pro12Ala and 23 SNPs that mapped to unique locations in and around *HNF4A*, 16 of which were chosen from the dbSNP database (rs2868093, rs6073418, rs717248, rs717247, rs736820, rs736822, rs736824, rs745975, rs736823, rs1885088, rs1885089, rs3212198, rs1028583, rs1028584, rs2273618, and rs911358) and 7 of which were selected on the basis of previous reports of association with type 2 diabetes in Finns (16) and/or Ashkenazim (17). SNPs were genotyped using a MassARRAY system (Sequenom, San Diego, CA) (26). Seven of 23 *HNF4A* SNPs were common to both IRASFS and BetaGene (rs4810424, rs1884613, rs1884614, rs2144908, rs2425637, rs2425640, and rs1885088). Based on 90 blinded dupli-

cate samples, the genotyping discrepancy rate was 0%, and overall genotype success rate was >90.0%.

Data analysis. In BetaGene, we calculated two measures of insulin response to glucose: 1) the difference between the 30' and fasting OGTT insulin concentrations (30' Δinsulin) and 2) the incremental area under the insulin curve during the first 10 min of the IVGTT (AIR). For both BetaGene and IRASFS, IVGTT glucose and insulin data were analyzed using the minimal model (MINMOD Millennium V5.18) to derive measures of glucose effectiveness (S_G) and S_I . The disposition index is computed as the product of S_I and AIR and measures β -cell compensation for insulin resistance (27).

For both studies, the observed genotype frequencies were assessed for deviation from Hardy-Weinberg equilibrium, and allele frequencies for each SNP were estimated using family data and MENDEL (V5.7). Linkage disequilibrium and haplotype block structure were assessed using Haploview V3.2 (28) and the method of Gabriel et al. (29). Quantitative trait data were statistically transformed to approximate univariate normality before analyses. The measured genotypes approach under a variance components framework was used to test SNP associations with continuous phenotypes and implemented using SOLAR (V2.1.4).

In BetaGene, because of underlying linkage disequilibrium among the nine *HNF4A* SNPs, we tested five of the *HNF4A* SNPs for association with type 2 diabetes-related quantitative traits under additive, dominant, and recessive genetic models. Because of the low frequency of the *PPARG2* Ala allele, association with the Pro12Ala variant was only tested under a dominant genetic model for Ala. We tested the interaction between rs2144908 and Pro12Ala for association with type 2 diabetes-related traits due to observed univariate effects of *HNF4A* rs2144908 and the previously reported associations with type 2 diabetes (16,17). The multiplicative interaction effect between *PPARG2* Pro12Ala and *HNF4A* rs2144908 was tested using a likelihood ratio test.

We had 80% power to detect an association between an SNP with 50% allele frequency (as observed for *HNF4A* rs2144908) that explains 1.7% of the variation in S_I , assuming an additive model and $\alpha = 0.05$. Similarly, we had 80% power to detect an association between an SNP with 10% allele frequency (as observed for *PPARG2* P12A) that explains 1.7% of the variation in S_I , assuming a dominant genetic model and $\alpha = 0.05$. Finally, assuming a multiplicative interaction between two SNPs as described above, we had 80% power to detect an interaction that explains at least 1.6% of the variation in S_I .

Because BetaGene families were ascertained through a proband with previous GDM, we corrected for ascertainment bias in each model by conditioning on the proband's phenotype value. All models were adjusted for age, sex, and, where appropriate, BMI. Results were similar when BMI was not included as a model covariate. Linear modeling results are reported as means and SD, adjusted for age, sex, and BMI. All other results are reported as unadjusted medians and interquartile ranges. All BetaGene P values for univariate tests of association between SNPs and quantitative traits are Bonferroni-adjusted for the number of SNPs ($n = 6$), models ($n = 1$ for *PPARG2* and $n = 3$ for *HNF4A*), and traits ($n = 10$; Table 1). Because our a priori hypothesis concerning variant interaction only included *PPARG2* Pro12Ala and *HNF4A* rs2144908, P values for tests of interaction effects are Bonferroni adjusted for the number of models and traits only. Statistical significance was defined as a corrected $P < 0.05$. Tests in the IRASFS are a priori hypotheses based on BetaGene results, thus IRASFS P values were not corrected for multiple testing.

RESULTS

We report results from 473 individuals in 89 BetaGene families with complete OGTTs and IVGTTs. The size of the proband generation (proband, siblings, and cousins) for each family ranged from 1 to 12 with a mean of 5.3. Descriptive characteristics of BetaGene subjects are shown in Table 1. In general, probands, siblings, and cousins were matched on age, although there was a tendency for cousins to be younger than probands and their siblings. The median BMI exceeded the threshold for overweight among all subjects, although siblings and cousins had a significantly lower BMI compared with probands ($P = 0.001$ and $P = 2.1 \times 10^{-5}$, respectively). The correlations among the quantitative traits are presented in the online appendix (Supplemental Table S1).

From the IRASFS, this report includes 490 individuals in 60 families from San Antonio and 496 individuals in 30 families from the San Luis Valley. The San Antonio sample

TABLE 1
Subject characteristics

	BetaGene				IRASFS	
	Proband	Siblings	Cousins	All	San Antonio	San Luis Valley
<i>n</i>	71	231	171	473	490	496
Men/women	0/71	84/147	79/92	163/310	191/299	213/283
Age (years)	34.7 (7.8)	33.8 (10.5)	32.7 (11.8)	33.8 (10.6)	38.8 (20.1)	40.0 (16.8)
BMI (kg/m ²)	31.3 (8.7)	29.0 (6.4)*	27.4 (6.4)*†	28.8 (7.0)	28.7 (8.2)	26.4 (6.8)‡
Fasting glucose (mmol/l)	5.4 (0.9)	5.1 (0.6)*	5.1 (0.6)*	5.2 (0.6)	5.1 (0.7)	5.1 (0.6)‡
2-h glucose (mmol/l)	8.4 (3.5)	7.3 (2.5)*	6.4 (2.2)*†	7.2 (2.6)	—	—
Fasting insulin (pmol/l)	76 (62)	49 (49)*	42 (42)*	49 (56)	78 (72)	66 (60)‡
2-h insulin (pmol/l)	660 (556)	431 (437)*	333 (403)*	424 (465)	—	—
30' Δinsulin (pmol/l)	431 (285)	379 (295)	410 (410)	399 (319)	—	—
S _G (×10 ⁻² min ⁻¹)	1.29 (0.42)	1.55 (0.71)*	1.74 (0.77)*	1.53 (0.74)	1.86 (1.00)	2.24 (1.02)‡
S _I (×10 ⁻³ min ⁻¹ per pmol/l)	2.17 (1.24)	2.62 (1.94)	2.94 (1.95)	2.65 (1.77)	2.47 (2.92)	3.17 (3.83)
AIR (pmol/l × 10 min)	3,467 (4,313)	4,280 (4,677)*	4,783 (6,050)*	4,340 (5,070)	5,506 (5,945)	6,262 (6,475)‡
Disposition index	7,890 (7,799)	11,401 (11,458)*	13,249 (10,654)*	11,409 (11,225)	14,315 (18,101)	19,898 (26,954)‡

Data are median (interquartile range). *Uncorrected $P < 0.05$ vs. probands after adjusting for age, sex, and BMI (where appropriate). †Uncorrected $P < 0.05$ vs. siblings after adjusting for age, sex, and BMI (where appropriate). ‡Uncorrected $P < 0.05$ vs. San Antonio after adjusting for age, sex, and BMI (where appropriate).

had a significantly higher mean BMI ($P = 0.0006$) and lower mean S_G, AIR, and disposition index (all $P < 0.005$) compared with San Luis Valley (Table 1).

Table 2 shows allele frequencies estimated from BetaGene subjects compared with frequencies for the IRASFS San Antonio and San Luis Valley subjects. The minor alleles reported for *HNF4A* SNPs in Finns (16) were used as reference alleles. In BetaGene, *PPARG2* Pro12Ala and each of the nine *HNF4A* SNPs had allele frequencies that differed considerably from those observed in Finns and Ashkenazim (16,17). The *PPARG2* Ala allele frequency was ~10% in Mexican Americans compared with ~17% in Finns (16). The observed frequencies of *HNF4A* rs4810424, rs1884613, rs1884614, and rs2144908 reference alleles were all estimated at roughly 50% compared with ~20% in the Finns and Ashkenazim (16,17). Allele frequency differences for other *HNF4A* variants were smaller, ranging from 7 to 18%. Allele frequency estimates from the IRASFS were consistent with those estimated in BetaGene for the seven SNPs common to both studies.

Figure 1A shows the pairwise linkage disequilibrium and haplotype block structure for the nine *HNF4A* SNPs in BetaGene. The four SNPs in the P2-promoter region,

rs4810424, rs1884613, rs1884614, and rs2144908, were in near perfect linkage disequilibrium ($D' = 1.0$, $r^2 \geq 0.98$), forming a single 10.7-kb haplotype block; SNPs rs6031551 and rs6031552 were also in strong linkage disequilibrium ($D' = 1.0$, $r^2 = 0.94$), forming an independent 80-bp haplotype block. We chose to test rs2144908 from the first haplotype block, rs6031551 from the second block, and the remaining SNPs (rs2425637, rs2425640, and rs1885088) for association with phenotypes of interest in BetaGene. A similar block structure was observed in the IRASFS using a combination of San Antonio and San Luis Valley subjects (Fig. 1B). The linkage disequilibrium structure using all 23 *HNF4A* SNPs genotyped in the IRASFS is presented as Supplemental Fig. 1.

Neither *PPARG2* Pro12Ala nor any of the five *HNF4A* SNPs alone showed significant evidence for association with type 2 diabetes-related quantitative traits in BetaGene after correction for multiple comparisons. However, without multiple comparisons correction, *HNF4A* rs2144908 showed marginal association with the disposition index under a dominant model, where subjects with at least one copy of the *HNF4A* A allele had a 14% higher adjusted mean disposition index compared with those

TABLE 2
SNP characteristics among BetaGene and IRASFS participants

	Chromosome	kb Position*	Reference allele†	Reference allele frequency‡		
				BetaGene	IRASFS	
					San Antonio	San Luis Valley
<i>PPARG2</i>						
Pro12Ala (rs1801282)	3	12368125	Ala	0.103	0.118	0.100
<i>HNF4A</i>						
rs4810424	20	42408437	C	0.500	0.459	0.481
rs1884613	20	42413829	G	0.501	0.451	0.481
rs1884614	20	42413933	T	0.499	0.456	0.481
rs2144908	20	42419131	A	0.503	0.453	0.472
rs6031551	20	42423128	C	0.116	—	—
rs6031552	20	42423208	A	0.107	—	—
rs2425637	20	42457463	T	0.608	0.640	0.622
rs2425640	20	42461451	A	0.202	0.139	0.149
rs1885088	20	42472454	A	0.199	0.169	0.238

*Based on National Center for Biotechnology Information build 36.1. †Minor allele for Finns in FUSION (16). ‡Allele frequency of BetaGene reference allele.

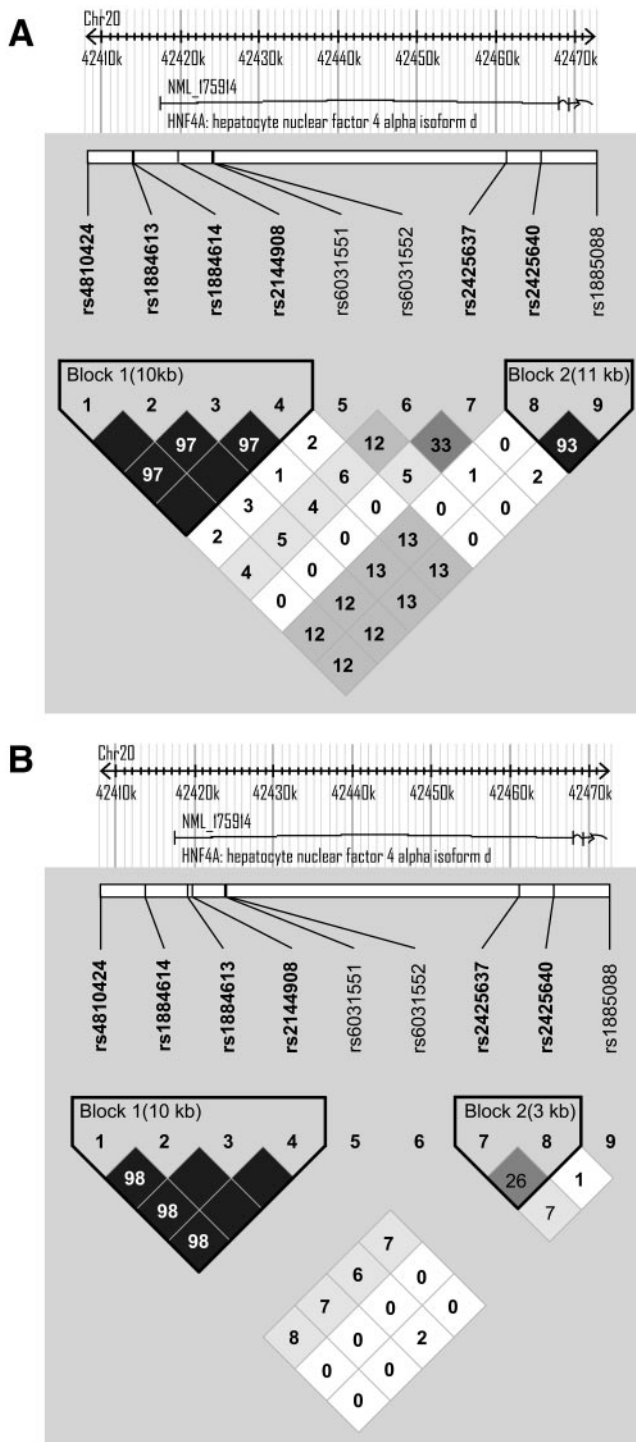


FIG. 1. *HNF4A* pairwise linkage disequilibrium and haplotype block structure. **A:** Linkage disequilibrium and haplotype block structure based on the nine SNPs genotyped in all BetaGene subjects. Haplotype blocks were determined using the method of Gabriel et al. (29) as implemented in Haploview V3.2. **B:** Linkage disequilibrium and haplotype block structure based on the seven SNPs genotyped in the IRASFS that were common to BetaGene. The linkage disequilibrium and haplotype block structure based on the combined San Antonio and San Luis Valley data are shown, because the results were nearly identical between the two samples. The linkage disequilibrium and block structure based on all 23 SNPs genotyped in the IRASFS is presented as Supplemental Fig. 1. Linkage disequilibrium is displayed as pairwise r^2 values, where white indicates $r^2 = 0$, varying shades of gray indicate $0 < r^2 < 1$, and black indicates $r^2 = 1$.

homozygous for the G allele ($13,493 \pm 2,911$ vs. $11,538 \pm 2,767$; uncorrected $P = 0.034$). Pro12Ala also showed marginal association with triglycerides under a dominant model, where subjects with at least one *PPARG2* Ala allele had a 12% lower adjusted mean triglyceride level compared with those homozygous for *PPARG2* Pro (0.90 ± 0.27 vs. 1.04 ± 0.25 mmol/l; uncorrected $P = 0.05$). Univariate association results for rs1801282 (*PPARG2* Pro12Ala) and rs2144908 (*HNF4A*) are presented in the online appendix (Supplemental Table S2).

Consistent with BetaGene, none of the SNPs tested in the IRASFS showed significant evidence for association with type 2 diabetes-related quantitative traits after multiple comparisons correction. *HNF4A* rs2144908 showed nominal association with S_1 (uncorrected $P = 0.024$) and disposition index (uncorrected $P = 0.047$) under an additive model in the San Antonio sample. Pro12Ala showed nominal association with S_1 in the San Luis Valley sample under a dominant genetic model (uncorrected $P = 0.01$) but no association with triglycerides.

Although none of the *PPARG2* or *HNF4A* variants individually showed significant association with diabetes-related quantitative traits, the multiplicative interaction between *PPARG2* Pro12Ala and *HNF4A* rs2144908 was significantly associated with S_1 ($P = 0.001$) and 2-h insulin ($P = 0.054$), assuming an additive genetic model for rs2144908 (Table 3). The interaction was also nominally associated with fasting insulin (uncorrected P value = 0.01) and AIR (uncorrected P value = 0.07), but these associations did not remain significant after correction for multiple comparisons.

Figure 2A shows the age, sex, and BMI adjusted mean S_1 in BetaGene stratified by *HNF4A* rs2144908 and *PPARG2* Pro12Ala genotypes. Mean S_1 increased progressively with each copy of the A allele for *HNF4A* rs2144908 among subjects with at least one copy of *PPARG2* Ala. In contrast, mean S_1 did not differ across *HNF4A* rs2144908 genotypes among *PPARG2* Pro homozygotes. Figure 2B shows the same interaction result under a recessive genetic model for the *HNF4A* rs2144908 A allele. Among subjects with at least one *PPARG2* Ala allele, subjects homozygous for *HNF4A* rs2144908 A allele had a 68% higher adjusted mean S_1 than those with at least one G allele ($P = 0.0001$). In contrast, among subjects homozygous for the *PPARG2* Pro allele, mean S_1 was not significantly different between individuals homozygous for the *HNF4A* rs2144908 A allele and those with a G allele ($P = 0.30$).

The interaction result for S_1 was replicated in the IRASFS San Antonio sample (additive $P = 0.056$, recessive $P = 0.018$; Table 4) but not in the San Luis Valley sample (Table 4; Fig. 3). The patterns observed in the San Antonio sample (Fig. 3A) were similar to those observed in BetaGene (compare with Fig. 2). Under an additive genetic model for *HNF4A* rs2144908 in BetaGene, mean S_1 increased by 40 and 44% with each copy of the A allele for *HNF4A* rs2144908 among subjects with at least one copy of the *PPARG2* Ala allele. No increase was seen among subjects homozygous for the *PPARG2* Pro allele. In the San Antonio sample from the IRASFS, the increases in mean S_1 with each copy of the *HNF4A* rs2144908 A allele were 29 and 16% among subjects with at least one copy of the *PPARG2* Ala allele, with no increase among subjects homozygous for *PPARG2* Pro (compare with Fig. 3A). Under a recessive genetic model for the *HNF4A* rs2144908 A allele, subjects with at least one *PPARG2* Ala allele and

TABLE 3
Trait association with interaction between *PPARG2* and *HNF4A* among BetaGene subjects

	<i>PPARG2</i> Pro12Ala genotype				<i>HNF4A</i> rs2144908 genotype				Interaction <i>P</i> value	
	A/A	A/G	G/G	A/A	A/G	G/G	A/A	G/G	Additive*	Recessive*
<i>n</i>	30	44	23	102	190	84				
BMI (kg/m ²)	26.3 (0.7)	27.4 (0.8)	27.8 (0.9)	27.8 (1.0)	27.8 (1.0)	27.9 (1.1)			1	1
Fasting glucose (mmol/l)	5.1 (0.2)	5.1 (0.3)	5.0 (0.2)	5.1 (0.2)	5.1 (0.2)	5.1 (0.2)			1	1
2-h glucose (mmol/l)	6.3 (0.6)	7.0 (0.9)	7.0 (0.8)	7.0 (0.8)	7.1 (0.8)	7.0 (0.8)			1	1
Fasting insulin (pmol/l)	36 (15)	57 (46)	79 (77)	53 (28)	59 (42)	54 (32)			0.636	0.294
2-h insulin (pmol/l)	279 (93)	467 (212)	625 (292)	405 (142)	413 (179)	383 (155)			0.054	0.039
30' Δinsulin (pmol/l)	334 (59)	432 (98)	588 (173)	406 (93)	412 (111)	396 (118)			0.222	0.021
S _G (×10 ⁻² min ⁻¹)	1.79 (0.18)	1.56 (0.22)	1.47 (0.21)	1.69 (0.19)	1.62 (0.21)	1.59 (0.20)			0.0011	0.0006
S _I (×10 ⁻³ min ⁻¹ per pmol/l)	3.92 (0.88)	2.73 (0.92)	1.95 (0.72)	2.74 (0.73)	2.71 (0.85)	2.89 (0.85)			0.969	1
AIR (pmol/l × 10 min)	4,480 (921)	5,198 (1,353)	6,832 (2,707)	5,230 (1,410)	5,060 (1,538)	4,567 (1,534)			1	1
Disposition index	16,113 (2,686)	13,026 (3,152)	12,426 (2,829)	13,561 (2,882)	12,742 (2,805)	12,257 (2,899)			1	1

Genotype-specific trait values are shown as mean (SD) adjusted for age, sex, and BMI (except in the case of BMI). The interaction *P* value is Bonferroni-corrected for 30 tests, as described in RESEARCH DESIGN AND METHODS. *Genetic model assumed for *HNF4A* rs2144908 in the interaction with *PPARG2*. *PPARG2* Pro12Ala was assumed to follow a dominant genetic model for Ala.

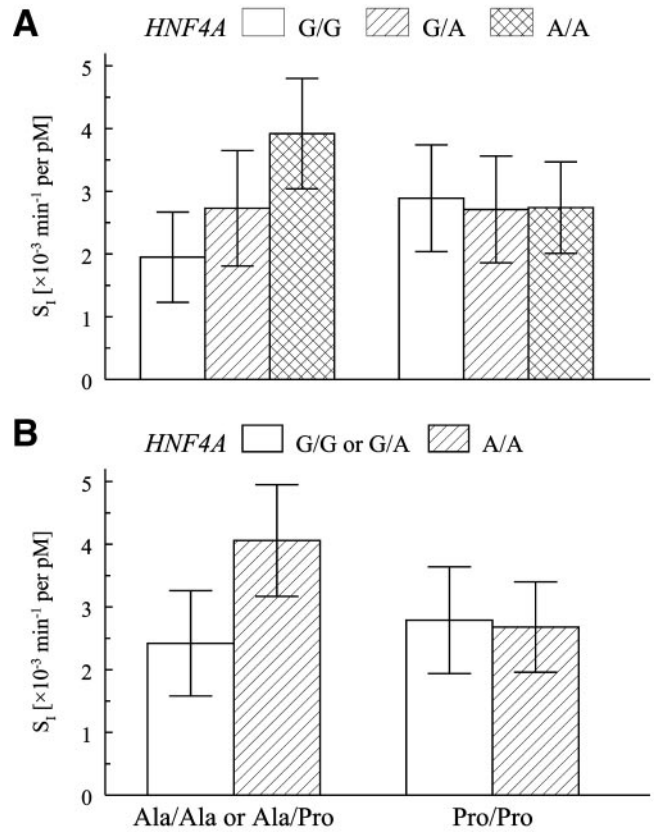


FIG. 2. Association with *S*₁ and the interaction between *PPARG2* and *HNF4A* among BetaGene subjects. *A*: Genotype-specific means \pm SD, adjusted for age, sex, and BMI stratified by *PPARG2* Pro12Ala and *HNF4A* rs2144908. Mean values were generated by computing the predicted value under the additive model and removing the transformation to maintain physiological interpretation. *A*: Values when *PPARG2* was assumed to follow a dominant genetic model for Ala and *HNF4A* rs2144908 was assumed to follow an additive genetic model for the A allele. The test of association for the interaction was significant (*P* = 0.0011). *B*: The same data when *PPARG2* remains modeled under a dominant genetic model, but *HNF4A* rs2144908 was assumed to follow a recessive genetic model for the A allele. The test for association was significant (*P* = 0.0006).

homozygous for rs2144908 A allele had adjusted mean *S*₁ values that were 31% higher than those with at least one G allele in the San Antonio sample (compare with Fig. 3A). The association between *S*₁ and the interaction between *PPARG2* and *HNF4A* was not observed in the San Luis Valley sample (Fig. 3B)

DISCUSSION

We found no significant association between *PPARG2* Pro12Ala or individual P2-promoter variants in *HNF4A* and type 2 diabetes-related quantitative traits in the BetaGene sample of Mexican-American families of a proband with previous GDM. By contrast, the interaction between *PPARG2* Pro12Ala and *HNF4A* rs2144908 was strongly associated with *S*₁. More importantly, this interaction was independently replicated in the San Antonio sample from the IRASFS but not in the San Luis Valley sample. In carriers of the *PPARG2* Ala allele, which has been reported to be protective from type 2 diabetes, *S*₁ increased with each copy of the A allele for *HNF4A* rs2144908, whereas *S*₁ remained low among all *HNF4A* genotypes in individuals with the “risk” *PPARG2* Pro allele. This pattern suggests that among individuals with the *PPARG2* Ala, the

TABLE 4
 S_1 association with interaction between *PPARG2* and *HNF4A* among IRASFS subjects

	<i>PPARG2</i> Pro12Ala genotype						Interaction <i>P</i> value	
	Ala/Pro or Ala/Ala			Pro/Pro				
	<i>HNF4A</i> rs2144908 genotype						Additive*	Recessive*
	A/A	A/G	G/G	A/A	A/G	G/G		
San Antonio								
<i>n</i>	32	63	23	75	187	104		
	3.65 (1.94)	3.15 (1.57)	2.45 (1.33)	2.71 (1.43)	2.58 (1.21)	2.42 (1.45)	0.056	0.018
San Luis Valley								
<i>n</i>	19	44	25	85	214	109		
	3.18 (1.69)	3.74 (1.74)	3.99 (2.04)	3.22 (1.58)	3.30 (1.69)	3.26 (1.73)	0.401	0.176

Genotype-specific trait values are shown as mean (SD) adjusted for age, sex, and BMI. *Genetic model assumed for *HNF4A* rs2144908 in the interaction with *PPARG2*. *PPARG2* Pro12Ala was assumed to follow a dominant genetic model for Ala.

A allele for *HNF4A* may afford some protection from insulin resistance but not in the presence of the G allele for *HNF4A* rs2144908 or *PPARG2* Pro. The reduced S_1 observed in *PPARG2* Pro homozygotes or in individuals with at least one *PPARG2* Ala and one *HNF4A* rs2144908 G allele may place them at increased risk for type 2 diabetes.

The interaction first observed in BetaGene was replicated in the San Antonio sample from the IRASFS but not in the San Luis Valley sample. There are several possible

explanations for the observed difference in results, including low statistical power or type 1 statistical error, ethnic admixture, and cryptic stratification. Although our power calculations indicated that we had sufficient power to detect the association in both studies, it is noteworthy that both BetaGene and the San Antonio sample from IRASFS had ~30 subjects who were homozygous for the *HNF4A* rs2144908 A allele and had at least one *PPARG2* Ala, whereas there were only 19 subjects with the same geno-

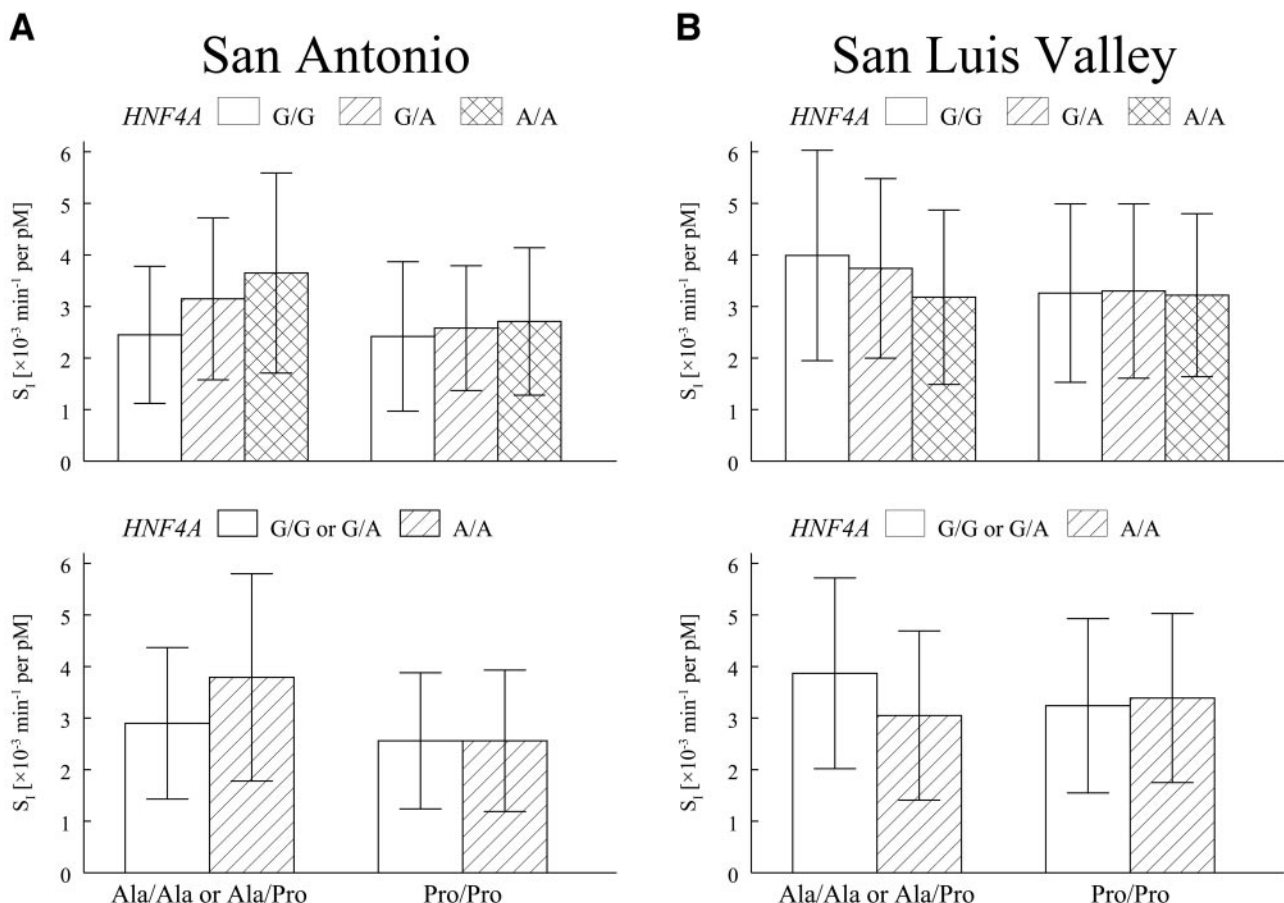


FIG. 3. Association with S_1 and the interaction between *PPARG2* and *HNF4A* among IRASFS subjects. **A**: Genotype-specific means \pm SD, adjusted for age, sex, and BMI stratified by *PPARG2* Pro12Ala and *HNF4A* rs2144908 for the San Antonio samples. **B**: The same data for the San Luis Valley samples. Mean values were generated by computing the predicted value under the additive model and removing the transformation to maintain physiological interpretation. Figures at the top of each panel show values when *PPARG2* was assumed to follow a dominant genetic model for Ala and *HNF4A* rs2144908 was assumed to follow an additive genetic model for the A allele. Figures at the bottom show the same data assuming a recessive genetic model for the *HNF4A* rs2144908 A allele. The test of association for the interaction was significant in the San Antonio samples but not in the San Luis Valley samples (see text for details).

type combination in the San Luis Valley sample. Replication in a larger sample would provide additional support for our observation, but we are not aware of additional genetic studies in Mexican Americans with frequently sampled IVGTT-derived measures of S_1 of equal or greater size than BetaGene or IRASFS. Within the individual analyses of the three samples, we do not believe cryptic stratification or ethnic admixture is contributing to our results. First, both BetaGene and IRASFS use family-based designs, which afford some protection against cryptic stratification, even in the absence of applying a family-based statistic like the quantitative transmission disequilibrium test. Second, BetaGene participants were required to have Mexican ancestry by self-reported birthplace going back two generations (compare with the online appendix), which should minimize admixture due to other Latino groups such as Central or South Americans.

However, the observed difference in outcome between BetaGene and San Antonio versus San Luis Valley could be due to differences in admixture. The two IRASFS Mexican-American samples differ substantially in terms of environment and relevant metabolic characteristics. First, San Antonio is an urban environment, whereas the San Luis Valley is a rural, high-elevation (~7,500 feet above sea level) environment. Second, the San Luis Valley families tend to be leaner and have better glucose homeostasis profiles, with significantly lower BMI and higher S_G , AIR, and disposition index, compared with the San Antonio sample (compare with Table 1). Although it is not possible to directly compare metabolic parameters between San Antonio and BetaGene participants because of differences in assays, these two groups are more similar compared with the San Luis Valley participants based on demographic parameters, e.g., age and BMI. This led us to analyze the two IRASFS samples separately and may explain the very similar results in BetaGene and San Antonio samples compared with the San Luis Valley sample. In addition, the ancestry of San Luis Valley Mexican Americans may differ from the urban Mexican Americans in San Antonio or BetaGene. Mexican Americans in the San Luis Valley tend to self-identify as “Spanish” (30), and admixture analysis suggests a large proportion of “Spanish” (~60%) and less Native American (~30%) admixture in this population (31). This is in contrast to recent estimates of 48% European and 40% Native American for Mexican Americans from Los Angeles (32) and even higher Native American admixture estimates for Southwestern Hispanics (33).

It is important to note that our analysis simply denotes a statistical interaction between variants and cannot be used to accurately characterize the underlying biology. Although additional studies will be required to characterize the underlying biology of this interaction, one can envision two general scenarios to explain how these two genes may interact to alter S_1 . The first presumes that both genes are expressed in the same tissues where they could interact directly (although there currently is no evidence that they do) or co-regulate transcription of genes in the same or interacting biochemical pathways. *PPARG2* is expressed in adipose tissue (7,8) where it regulates a number of genes (34), and the Pro variant could result in increased activation of genes in pathways, such as adipogenesis, that contribute to lower S_1 (9). *HNF4A* regulates a large network of genes in both the pancreas and liver (14). rs2144908 lies near the P2-promoter of *HNF4A*, which is believed to be primarily active in pancreatic β -cells and

thought to regulate insulin secretion (35). However, there is evidence of *PPARG2* expression in liver with development of hepatic steatosis, a condition typically accompanied by obesity, impaired glucose tolerance, and type 2 diabetes (36–38). Also, studies by Thomas et al. show there may be low-level activity of the *HNF4A* P2-promoter in hepatocytes (35). Thus, the presence of *PPARG2* Pro and *HNF4A* rs2144908 G allele could result in dysregulation of hepatic gene transcription, leading to hepatic insulin resistance and thereby reducing S_1 .

The other possibility is that these two genes may act independently in different tissues, but the net integrated physiological effect of these genes alters S_1 . *PPARG2* Pro12Ala showed marginal association with triglyceride levels in BetaGene, with individuals homozygous for Pro having modestly elevated triglycerides. Stumvoll et al. (39) reported an association between the Ala allele and increased S_1 compared with the Pro/Pro genotype, due to enhanced insulin action on the suppression of lipolysis resulting in decreased release of free fatty acids. This suggests an effect of *PPARG2* Pro allele on adipose tissue that could result in elevated fatty acid flux, which could contribute to impaired ability to suppress hepatic glucose output (40). As noted above, variation in *HNF4A* rs2144908 could disrupt gene transcription within the liver, which could further contribute to hepatic insulin resistance.

To better understand the potential mechanisms underlying this interaction, we applied the prioritizing disease genes by analysis of common elements (PDG-ACE) algorithm (online appendix). When applied to our *PPARG2*-*HNF4A* interaction, “triglyceride” was identified as a common, over-represented term (Bonferroni-corrected $P = 0.016$) in the annotation of these genes. Additional examination showed literature citing both *PPARG2* and *HNF4A* as potential regulators of human microsomal triglyceride transfer protein (*MTTP*) (41,42), which is involved in lipoprotein assembly in the intestine and VLDL in the liver (43). Expression of *MTTP* is elevated in type 2 diabetes (43), which can lead to dyslipidemia. Variation in *MTTP* has also been shown to be associated with type 2 diabetes and postprandial insulin levels (44). Thus, the interaction between *PPARG2* and *HNF4A*, as described under the above scenarios, may work through *MTTP* to change triglyceride levels, which could alter S_1 .

In conclusion, we did not observe association between diabetes-related quantitative traits and either *PPARG2* Pro12Ala or variation in the *HNF4A* promoter region in Mexican-American families of a proband with previous GDM. We did observe a strong association between S_1 and the interaction between *PPARG2* Pro12Ala and *HNF4A* rs2144908 in BetaGene. This interaction was independently replicated in the San Antonio subjects from the IRASFS but not in the San Luis Valley sample. The characteristics of the interaction suggest that having *PPARG2* Pro leads to relatively low S_1 regardless of *HNF4A* genotype, while the impact of the type 2 diabetes “protective” *PPARG2* Ala depends at least in part on the *HNF4A* genotype. The biological nature of this interaction requires further investigation.

ACKNOWLEDGMENTS

T.E.F. is supported by an American Diabetes Association (ADA) Junior Faculty Award. T.A.B. is partly supported by an ADA Distinguished Clinical Scientist Award. R.M.W. is

partly supported by ADA Research Grant 1-05-RA-140. The BetaGene study is supported by National Institutes of Health (NIH) Grant DK-61628 awarded to T.A.B. A portion of the work for BetaGene was conducted in a facility constructed with support from Research Facilities Improvement Program Grant Number C06 (RR-10600-01, CA-62528-01, and RR-14514-01) from the National Center for Research Resources. The IRAS Family Study (IRASFS) is supported by NIH Grants HL-60944-02, HL-61210-02, HL-61019-02, HL-60894, and HL-60931-02. The Prioritizing Disease Genes by Analysis of Common Elements (PDG-ACE) project is supported by NIH Grant U54-DA-021519.

We thank the families who volunteered to participate in the BetaGene study and IRASFS. We also gratefully acknowledge the tremendous dedication and work performed by the staff for both studies. The BetaGene study also acknowledges the support of the University of Southern California General Clinical Research Center (M01-RR-00043).

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