

Evidence of Interaction Between Peroxisome Proliferator-Activated Receptor- γ 2 (*PPARG2*) and Hepatocyte Nuclear Factor-4 α (*HNF4A*) Contributing to Variation in Insulin Sensitivity in Mexican Americans

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Running Title: *PPARG2* and *HNF4A* Interact to Alter S_I

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ABSTRACT

Objective: We hypothesized that interaction between PPARG2 Pro12Ala and variants in the promoter region of HNF4A are be associated with type 2 diabetes (T2D)-related quantitative traits (QT) in Mexican-American families of a proband with previous gestational diabetes mellitus (GDM).

Research Design and Methods: The BetaGene project genotyped PPARG2 Pro12Ala and 9 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 (OGTT) and intravenous (IVGTT) glucose tolerance tests.

Results: Neither PPARG2 Pro12Ala nor any of the 9 HNF4A SNPs were independently associated with T2D-related QTs. However, the interaction between PPARG2 Pro12Ala and HNF4A rs2144908 was significantly associated with both insulin sensitivity (S_I) (Bonferroni p=0.0006) and 2-hr insulin (Bonferroni p=0.039). Subjects with at least one PPARG2 Ala allele and homozygous for HNF4A rs2144908 A allele had 40% higher S_I compared to 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 IRAS Family Study (p=0.018) in their San Antonio sample (n=484) where subjects with at least one *PPARG2* Ala allele and homozygous for *HNF4A* rs2144908 A allele had a 29% higher S_I compared to 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 T2D.

Mexican American women with previous GDM exhibit significant β -cell dysfunction and are at high risk for developing T2D (1-3). Moreover, their risk for future T2D 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, DI), 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 T2D (9-12), as well as 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 T2D in Finns (16) and Ashkenazi Jews (17); some subsequent studies have replicated the association (18;19), while others have not (20;21). In Finns, variation in the P2-promoter region was also associated with measures of insulin secretion and β -cell

function in non-diabetic offspring of patients with T2D (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 T2D-related QTs in BetaGene. We then tested our positive results in two similarly phenotyped Mexican American samples from the Insulin Resistance Atherosclerosis Family Study (IRAS FS), one from San Antonio (SA), Texas and the other from the San Luis Valley (SLV) in Colorado (22).

METHODS

Subject Recruitment. Subject recruitment for BetaGene is on-going and is briefly described in the Supplemental Materials (available at <http://diabetes.diabetesjournals.org>). 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 mM) from families of a proband with GDM diagnosed within the previous 5 years who have available for study either 2 non-diabetic siblings and 3 non-diabetic 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.

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

All protocols for BetaGene and the IRAS FS were approved by the Institutional Review Boards of participating institutions and all

participants provided written informed consent prior to 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 DEXA 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, while parents, uncles, aunts, spouses, and offspring had only a physical exam, fasting glucose measurement, and DNA collection.

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

Assays. In both BetaGene and IRAS FS, 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 (TOSOH) that has <0.1% cross-reactivity with proinsulin and intermediate split products, while in IRAS FS 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* SNPs showing evidence for association with T2D 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 9 SNPs

were obtained. SNP genotyping was performed using the Applied Biosystems, Inc. (ABI) TaqMan system (25). Genotyping assays were either selected through ABI's "Assays on Demand" database (<http://myscience.appliedbiosystems.com/navigation/mysciapplications.jsp>) or custom designed using ABI's "Assays by Design" service. Based upon 22 blinded duplicate samples, the discrepancy rate for genotyping was 0% and overall genotype success rate was >97.6%.

IRAS FS 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 T2D 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 IRAS FS and BetaGene (rs4810424, rs1884613, rs1884614, rs2144908, rs2425637, rs2425640, and rs1885088). Based upon 90 blinded duplicate 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: a) the difference between the 30' and fasting OGTT insulin concentrations (30' Dinsulin) and b) the incremental area under the insulin curve during the first 10 min of the IVGTT (acute insulin response, AIR). For both BetaGene and IRAS FS, 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 DI is computed as the product of

S_I and AIR and measures b-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 (LD) and haplotype block structure were assessed using Haploview V3.2 (28) and the method of Gabriel (29). QT data were statistically transformed to approximate univariate normality prior to analyses. The measured genotypes approach under a variance components framework was used to test SNP associations with continuous phenotypes and implemented using SOLAR (V.2.1.4).

In BetaGene, due to underlying LD among the 9 *HNF4A* SNPs, we tested 5 of the *HNF4A* SNPs for association with T2D-related QTs under additive, dominant, and recessive genetic models. Due to 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 T2D-related traits due to observed univariate effects of *HNF4A* rs2144908, as well as the previously reported associations with T2D (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 a 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 a 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 and gender and, where appropriate, BMI. Results were similar when BMI was not included as a model covariate. Linear modeling results are reported as means and standard deviation, adjusted for age, gender 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 QTs are Bonferroni-adjusted for the number of SNPs ($n=6$), models ($n=1$ for *PPARG2*, $n=3$ for *HNF4A*) and traits ($n=10$; Table I). 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 IRAS FS are *a priori* hypotheses based on BetaGene results, thus IRAS FS 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 (probands, 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 I. 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 to

probands ($p=0.001$ and $p=2.1\times 10^{-5}$ respectively). The correlations among the QTs are presented in the supplementary material (Table S1).

From the IRAS FS, this report includes 490 individuals in 60 families from SA and 496 individuals in 30 families from the SLV. The SA sample had a significantly higher mean BMI ($p=0.0006$) and lower mean S_G, AIR and DI (all $p<0.005$) compared to SLV (Table I).

Table II shows allele frequencies estimated from BetaGene subjects compared to frequencies for the IRAS FS SA and SLV subjects. The minor alleles reported for *HNF4A* SNPs in Finns (16) were used as reference alleles. In BetaGene, *PPARG2* Pro12Ala and each of the 9 *HNF4A* SNPs had allele frequencies that differed considerably from those observed in Finns and Ashkenazim (16;17). The *PPARG2* Ala allele frequency was approximately 10% in Mexican Americans compared to ~17% in Finns (16). The observed frequencies of *HNF4A* rs4810424, rs1884613, rs1884614, and rs2144908 reference alleles were all estimated at roughly 50% compared to ~20% in the Finns and Ashkenazim (16;17). Allele frequency differences for other *HNF4A* variants were smaller, ranging from 7-18%. Allele frequency estimates from the IRAS FS were consistent with those estimated in BetaGene for the seven SNPs common to both studies.

Figure 1A shows the pair-wise LD 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 LD ($D'=1.0$, $r^2\geq 0.98$), forming a single 10.7 kb haplotype block; SNPs rs6031551 and rs6031552 were also in strong LD ($D'=1.0$, $r^2=0.94$) 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 IRAS FS using a combination of SA and SLV subjects (Figure 1B). The LD structure using all 23 *HNF4A* SNPs genotyped in the IRAS FS is presented as Supplementary Figure 1.

Neither *PPARG2* Pro12Ala nor any of the 5 *HNF4A* SNPs alone showed significant evidence for association with T2D-related QTs in BetaGene after correction for multiple comparisons. However, without multiple comparisons correction, *HNF4A* rs2144908 showed marginal association with DI under a dominant model, where subjects with at least one copy of the *HNF4A* A allele had a 14% higher adjusted mean DI compared to those homozygous for the G allele (13493 ± 2911 vs. 11538 ± 2767 ; 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 to those homozygous for *PPARG2* Pro (0.90 ± 0.27 vs. 1.04 ± 0.25 mM; uncorrected $p=0.05$). Univariate association results for rs1801282 (*PPARG2* Pro12Ala) and rs2144908 (*HNF4A*) are presented in the supplementary materials (Table S2).

Consistent with BetaGene, none of the SNPs tested in the IRAS FS showed significant evidence for association with T2D-related QTs after multiple comparisons correction. *HNF4A* rs2144908 showed nominal association with S₁ (uncorrected $p=0.024$) and DI (uncorrected $p=0.047$) under an additive model in the SA sample. Pro12Ala showed nominal association with S₁ in the SLV 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 QTs, the

multiplicative interaction between *PPARG2* Pro12Ala and *HNF4A* rs2144908 was significantly associated with S_I (p=0.001) and 2-hr insulin (p=0.054) assuming an additive genetic model for rs2144908 (Table III). 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, gender and BMI adjusted mean S_I in BetaGene stratified by *HNF4A* rs2144908 and *PPARG2* Pro12Ala genotypes. Mean S_I 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_I 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 *HNF4A* rs2144908 A allele had a 68% higher adjusted mean S_I than those with at least one G allele (p=0.0001). In contrast, among subjects homozygous for the *PPARG2* Pro allele, mean S_I 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_I was replicated in the IRAS FS SA sample (additive p=0.056, recessive p=0.018; Table IV), but not in the SLV sample (Table IV and Figure 3). The patterns observed in the SA sample (Figure 3A) were similar to those observed in BetaGene (cf. Figure 2). Under an additive genetic model for *HNF4A* rs2144908 in BetaGene, mean S_I 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 SA sample from the IRAS FS, the increases in mean S_I 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 (cf. Figure 3A). Under a recessive genetic model for the *HNF4A* rs2144908 A allele, subjects with at least one *PPARG2* Ala allele and homozygous for rs2144908 A allele had adjusted mean S_I values that were 31% higher than those with at least one G allele in the San Antonio sample (cf. Figure 3A). The association between S_I and the interaction between *PPARG2* and *HNF4A* was not observed in the SLV sample (Figure 3B).

DISCUSSION

We found no significant association between *PPARG2* Pro12Ala or individual P2-promoter variants in *HNF4A* and T2D-related QTs 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_I. More importantly, this interaction was independently replicated in the SA sample from the IRAS FS, but not in the SLV sample. In carriers of the *PPARG2* Ala allele, which has been reported to be protective from T2D, S_I increased with each copy of the A allele for *HNF4A* rs2144908, whereas S_I remained low among all *HNF4A* genotypes in individuals with the “risk” *PPARG2* Pro allele. This pattern suggests that among individuals with *PPARG2* Ala, the 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_I 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 T2D.

The interaction first observed in BetaGene was replicated in the SA sample from the IRAS FS, but not in the SLV 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 we had sufficient power to detect the association in both studies, it is noteworthy that both BetaGene and the SA sample from IRAS FS had ~30 subjects who were homozygous for the *HNF4A* rs2144908 A allele and had at least one *PPARG* Ala, while there were only 19 subjects with the same genotype combination in the SLV 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 FSIGT-derived measures of S_I of equal or greater size than BetaGene or IRAS FS. 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 IRAS FS use family-based designs, which afford some protection against cryptic stratification even in the absence of applying a family-based statistic like the Q-TDT. Second, BetaGene participants were required to have Mexican ancestry by self-reported birthplace going back two generations (*cf.* Supplemental Materials), which should minimize admixture due to other Latino groups such as Central or South Americans.

However, the observed difference in outcome between BetaGene and SA versus SLV could be due to differences in admixture. The two IRAS FS Mexican American samples differ substantially in terms of environment and relevant metabolic characteristics. First, SA is an urban environment while the SLV is a rural, high-

elevation (~7,500 feet above sea level) environment. Second, the SLV families tend to be leaner and have better glucose homeostasis profiles, with significantly lower BMI and higher S_G , AIR and DI, compared to the SA sample (*cf.* Table I). Although it is not possible to directly compare metabolic parameters between SA and BetaGene participants due to differences in assays, these two groups are more similar compared to the SLV participants based on demographic parameters, *e.g.*, age, BMI. This led us to analyze the two IRAS FS samples separately and may explain the very similar results in BetaGene and SA samples compared to the SLV sample. In addition, the ancestry of SLV Mexican Americans may differ from the urban Mexican Americans in SA or BetaGene. Mexican Americans in the SLV 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_I . 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 of 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_I (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 T2D (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_I.

The other possibility is that these two genes may act independently in different tissues, but the net integrated physiologic effect of these genes alters S_I. *PPARG2* Pro12Ala showed marginal association with triglyceride levels in BetaGene, with individuals homozygous for Pro having modestly elevated triglycerides. Stumvoll *et al.* reported an association between the Ala allele and increased S_I compared to the Pro/Pro genotype, due to enhanced insulin action on the suppression of lipolysis resulting in decreased release of free fatty acids (39). 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 (*cf.* Supplemental Materials). 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 T2D (43), which can lead to dyslipidemia. Variation in *MTTP* has also been shown to be associated with T2D and post-prandial 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_I.

In conclusion, we did not observe association between diabetes-related QTs 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_I and the interaction between *PPARG2* Pro12Ala and *HNF4A* rs2144908 in BetaGene. This interaction was independently replicated in the SA subjects from the IRAS FS, but not in the SLV sample. The characteristics of the interaction suggest that having *PPARG2* Pro leads to relatively low S_I regardless of *HNF4A* genotype, while the impact of the T2D “protective” *PPARG2* Ala depends at least in part on *HNF4A* genotype. The biologic nature of this interaction requires further investigation.

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TABLE 1. Subject Characteristics

Values are median (interquartile range)						
	BetaGene				IRAS FS	
	Probands (n=71)	Siblings (n=231)	Cousins (n=171)	All (n=473)	San Antonio (n=490)	San Luis Valley (n=496)
Male / Female	0/71	84/147	79/92	163/310	191/299	213/283
Age [yrs.]	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 [mM]	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-hour Glucose [mM]	8.4 (3.5)	7.3 (2.5)*	6.4 (2.2)*†	7.2 (2.6)	--	--
Fasting Insulin [pM]	76 (62)	49 (49)*	42 (42)*	49 (56)	78 (72)	66 (60)‡
2-hour Insulin [pM]	660 (556)	431 (437)*	333 (403)*	424 (465)	--	--
30' ΔInsulin [pM]	431 (285)	379 (295)	410 (410)	399 (319)	--	--
S_G [$\times 10^{-2}$ 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 [$\times 10^{-3}$ min ⁻¹ per pM]	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 [pM $\times 10$ min]	3467 (4313)	4280 (4677)*	4783 (6050)*	4340 (5070)	5506 (5945)	6262 (6475)‡
Disposition Index	7890 (7799)	11401 (11458)*	13249 (10654)*	11409 (11225)	14315 (18101)	19898 (26954)‡

* uncorrected $p < 0.05$ vs. probands after adjusting for age, gender and BMI (where appropriate).

† uncorrected $p < 0.05$ vs. siblings after adjusting for age, gender and BMI (where appropriate).

‡ uncorrected $p < 0.05$ vs. San Antonio after adjusting for age, gender and BMI (where appropriate).

TABLE 2. SNP Characteristics among BetaGene and IRAS Family Study Participants

SNP	Chromosome	Kb Position*	Reference [†] Allele	Reference Allele Frequency [‡]		
				BetaGene	IRAS FS San Antonio	IRAS FS 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 NCBI build 36.1

[†] Minor Allele Frequencies for Finns in FUSION (Reference #16)[‡] Allele Frequency of BetaGene Reference Allele

TABLE 3. Trait Association with Interaction Between *PPARG2* and *HNF4A*, among BetaGene Subjects

Genotype-specific trait values are shown as mean (SD) adjusted for age, gender and BMI (except in the case of BMI). The interaction p-value is Bonferroni-corrected for 30 tests, as described in the Methods.

Trait	<i>PPARG2</i> Pro12Ala Genotype						Interaction p-value Additive* Recessive*	
	Ala/Pro or Ala/Ala			Pro/Pro				
	<i>HNF4A</i> rs2144908 Genotype							
	A/A (n=30)	A/G (n=44)	G/G (n=23)	A/A (n=102)	A/G (n=190)	G/G (n=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 [mM]	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-hour Glucose [mM]	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 [pM]	36 (15)	57 (46)	79 (77)	53 (28)	59 (42)	54 (32)	0.636	0.294
2-hour Insulin [pM]	279 (93)	467 (212)	625 (292)	405 (142)	413 (179)	383 (155)	0.054	0.039
30' D Insulin [pM]	334 (59)	432 (98)	588 (173)	406 (93)	412 (111)	396 (118)	0.222	0.021
S _G [$\times 10^{-2}$ 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)	1	1
S _I [$\times 10^{-3}$ min ⁻¹ per pM]	3.92 (0.88)	2.73 (0.92)	1.95 (0.72)	2.74 (0.73)	2.71 (0.85)	2.89 (0.85)	0.0011	0.0006
AIR [pM $\times 10$ min]	4480 (921)	5198 (1353)	6832 (2707)	5230 (1410)	5060 (1538)	4567 (1534)	0.969	1
Disposition Index	16113 (2686)	13026 (3152)	12426 (2829)	13561 (2882)	12742 (2805)	12257 (2899)	1	1

* Refers to genetic model assumed for *HNF4A* rs2144908 in the interaction with *PPARG2*. *PPARG2* Pro12Ala was assumed to follow a dominant genetic model for Ala.

TABLE 4. S₁ Association with Interaction Between PPARG2 and HNF4A, among IRAS FS Subjects

Genotype-specific trait values are shown as mean (SD) adjusted for age, gender and BMI.

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

* Refers to genetic model assumed for *HNF4A* rs2144908 in the interaction with *PPARG2*. *PPARG2* Pro12Ala was assumed to follow a dominant genetic model for Ala.

FIGURE LEGENDS

Figure 1. *HNF4A* Pair-wise Linkage Disequilibrium and Haplotype Block Structure. Panel A shows LD and haplotype block structure based upon the 9 SNPs genotyped in all BetaGene subjects. Haplotype blocks were determined using the method of Gabriel as implemented in Haploview V3.2. Panel B shows LD and haplotype block structure based upon the 7 SNPs genotyped in the IRAS FS that were common to BetaGene. The block LD and haplotype block structure based on the combined SA and SLV data are shown, as the results were nearly identical between the two samples. The LD and block structure based on all 23 SNPs genotyped in the IRAS FS is presented as Supplementary Figure 1. LD is displayed as pair-wise r^2 values, where white indicates $r^2 = 0$, varying shades of grey indicate $0 < r^2 < 1$, and black indicates $r^2 = 1$.

Figure 2. Association with S_I and the Interaction Between *PPARG2* and *HNF4A* among BetaGene subjects. Panel A shows genotype-specific mean±SD, adjusted for age, gender, 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 physiologic interpretation. Panel A shows 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). Panel B shows the same data when *PPARG2* remains modeled under a dominant 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).

Figure 3. Association with S_I and the Interaction Between *PPARG2* and *HNF4A* among IRAS FS subjects. Panel A shows genotype-specific mean±SD, adjusted for age, gender, and BMI stratified by *PPARG2* Pro12Ala and *HNF4A* rs214408 for the San Antonio samples. Panel B shows 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 physiologic interpretation. Figures at the top of each panel shows 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).

FIGURE 1

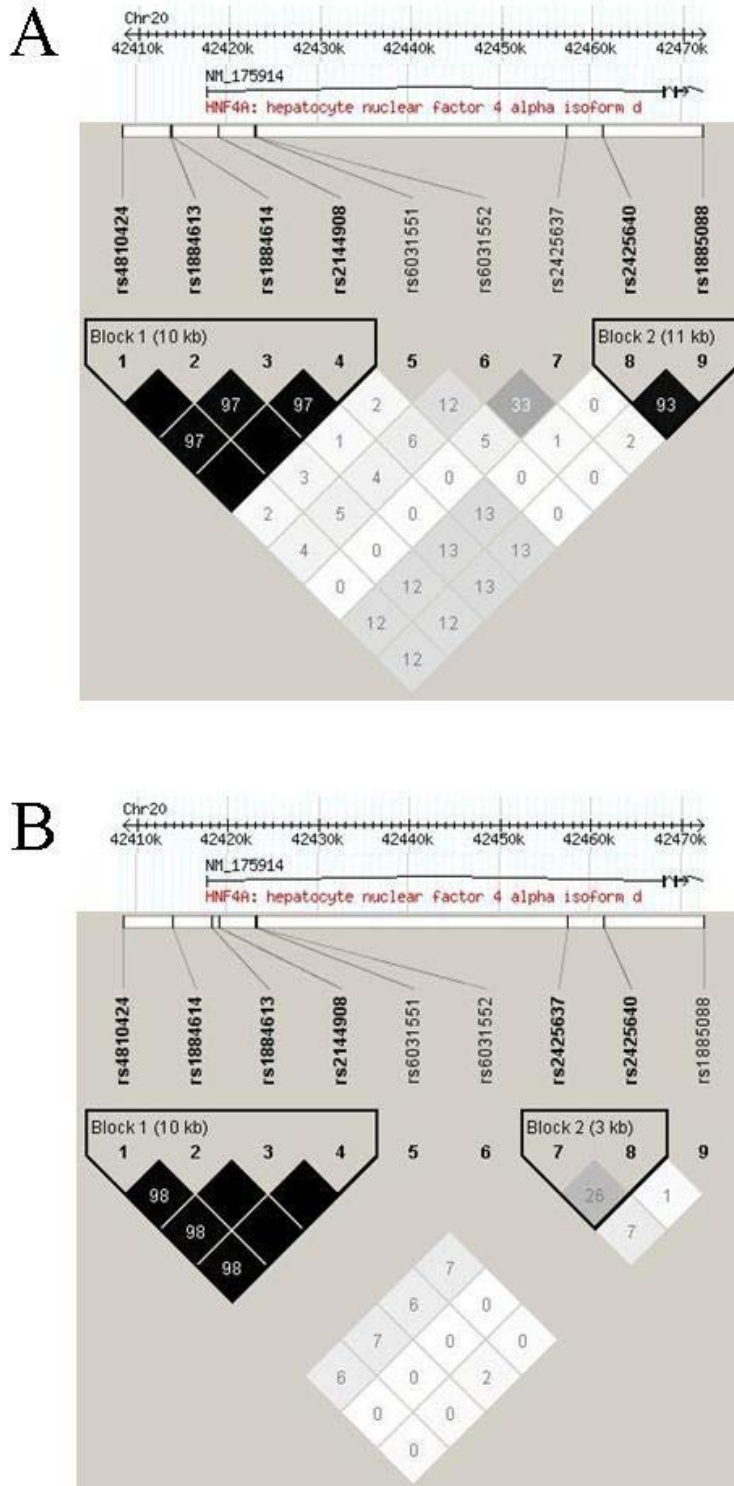


FIGURE 2

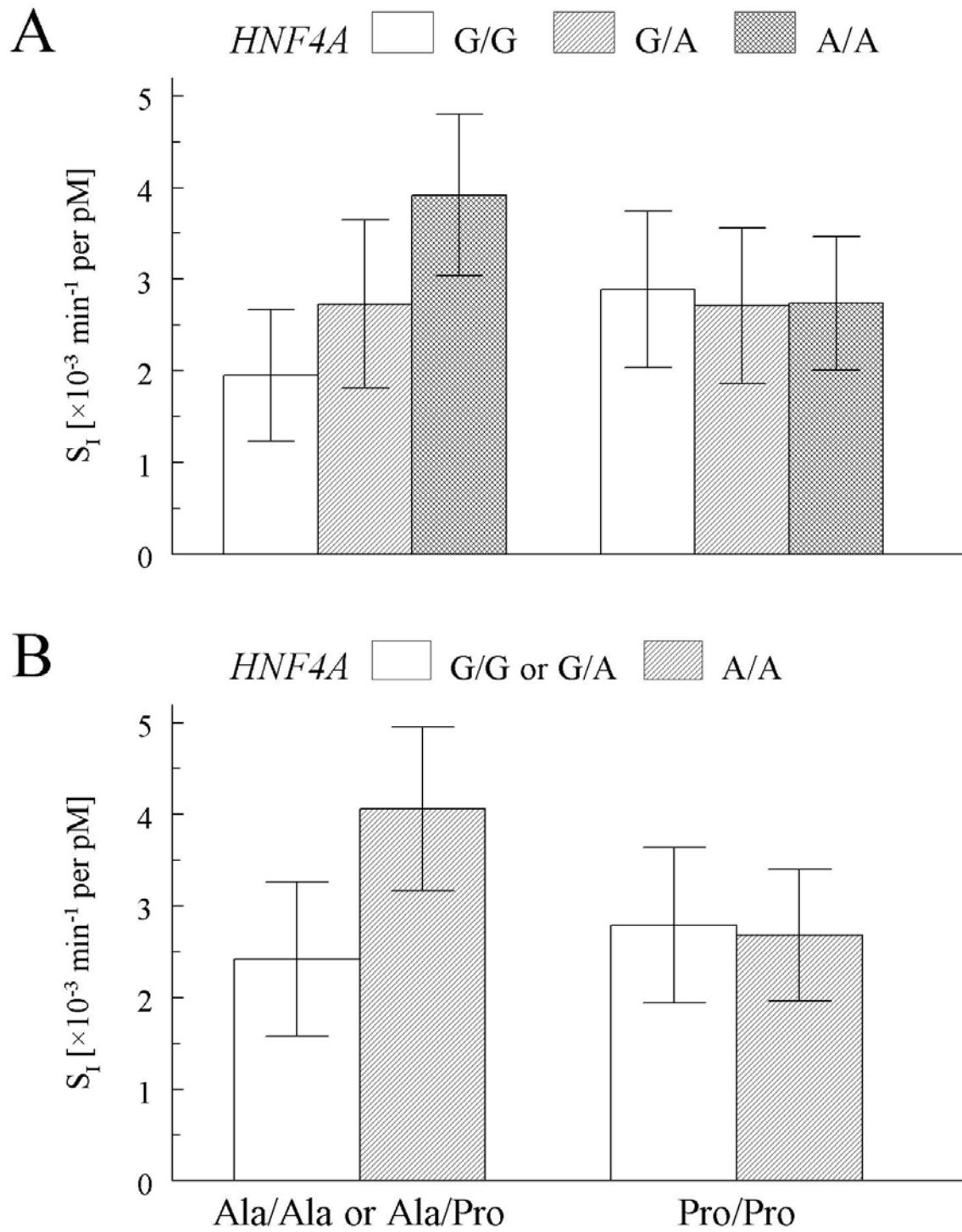


FIGURE 3

