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EFFECTS OF THE TYPE 2 DIABETES-ASSOCIATED *PPARG* P12A POLYMORPHISM ON PROGRESSION TO DIABETES AND RESPONSE TO TROGLITAZONE †

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Abstract

Context—The common P12A polymorphism in *PPARG* (a target for thiazolidinedione medications) has been consistently associated with type 2 diabetes.

Objective—We examined whether *PPARG* P12A affects progression from impaired glucose tolerance (IGT) to diabetes, or responses to preventive interventions (lifestyle, metformin or troglitazone versus placebo).

Patients—3,548 Diabetes Prevention Program participants.

Design—We performed Cox regression analysis using genotype at *PPARG* P12A, intervention, and their interactions as predictors of diabetes incidence. We also genotyped five other *PPARG* variants implicated in the response to troglitazone and assessed their effect on insulin sensitivity at one year.

Results—Consistent with prior cross-sectional studies, P/P homozygotes at *PPARG* P12A appeared more likely to develop diabetes than alanine carriers (hazard ratio 1.24, 95% CI 0.99–1.57, *P*=0.07),

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with no interaction of genotype with intervention. There was a significant interaction of genotype with body mass index (BMI) and waist circumference (*P*=0.03 and 0.002 respectively), with the alanine allele conferring less protection in more obese individuals. Neither *PPARG* P12A nor five other variants significantly affected the impact of troglitazone on insulin sensitivity in 340 participants at one year.

Conclusions—The proline allele at *PPARG* P12A increases risk for diabetes in persons with IGT, an effect modified by BMI. In addition, *PPARG* P12A has little or no effect on the beneficial response to troglitazone.

Keywords

Diabetes; *PPARG*; polymorphism

INTRODUCTION

The peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear hormone receptor preferentially expressed in adipose tissue (1). Activation by its ligand causes it to heterodimerize with the retinoid X receptor, bind specific DNA elements and induce a transcriptional cascade that leads to adipocyte differentiation and increased sensitivity to insulin. The PPARγ molecule is now recognized as the cognate receptor for thiazolidinediones (2). A proline → alanine change in codon 12 of its gene *PPARG* (P12A) has been reproducibly associated with a decreased risk for type 2 diabetes (3–12); the proline allele confers a ~20% increased risk under a recessive model. Because of its high frequency in the population, the population attributable risk of this variant nears 25% (4). Although some studies have not achieved statistical significance in their attempt to replicate this finding (13–21), most of them report consistent odds ratios (OR) with overlapping 95% confidence intervals, such that a meta-analysis of all published evidence yields a combined *P* value that achieves genome-wide significance (22). How this molecular change impairs protein function and leads to an increased risk of type 2 diabetes has not been fully elucidated (23).

The risk of type 2 diabetes conferred by *PPARG* P12A has also been evaluated prospectively. The Finnish Diabetes Prevention Study (24), which randomized 522 subjects with impaired glucose tolerance (IGT) to either placebo or a lifestyle intervention, reported a two-fold increase in risk of developing type 2 diabetes among alanine carriers in the placebo arm when compared to P/P homozygotes, a result which seemed to contradict the sizeable body of cross-sectional literature described above. On the other hand, the much larger Botnia Prospective Study (N=2,293) documented a hazard ratio (HR) for developing diabetes of 1.7 among P/P homozygotes, a result which was statistically significant (25). Different ascertainment schemes (IGT vs a population sample) and analytical methods (logistic regression vs Cox proportional hazards analysis) may explain some but not all of these discrepancies.

In addition to its role in increasing risk of type 2 diabetes, the P12A variant may also affect therapeutic response; if so, its putative impact on preventive interventions might have clinical utility. In support of this concept, two studies have examined the effect of *PPARG* P12A on response to thiazolidinediones (26,27). Blüher and colleagues treated 131 subjects with pioglitazone for 26 weeks; the proportion of responders (defined as >15% decrease in HbA_{1C} levels and/or >20% decrease in fasting blood glucose when compared to baseline after 12 or 26 weeks of pioglitazone) did not differ between P/P homozygotes and alanine carriers (26). Snitker and colleagues examined 93 Hispanic women with a previous history of gestational diabetes enrolled in the Troglitazone in Prevention of Diabetes (TRIPOD) study, and obtained intravenous glucose tolerance tests before and three months after treatment with

troglitazone; genotype at *PPARG* P12A did not explain the variability in insulin sensitivity observed among these women (27).

It is possible that these studies were underpowered or that other variants in *PPARG* may account for the differential therapeutic response. To examine the second possibility, the TRIPOD investigators genotyped a set of 131 common *PPARG* variants in the same group of 93 Hispanic women, and reported that eight *PPARG* polymorphisms were associated with response to troglitazone, defined as an overrepresentation of the minor allele in the upper two tertiles of insulin sensitivity (S_I during IVGTT) after three months of troglitazone treatment (28). Two of these SNPs (rs4135263 and rs1152003) also showed nominal associations with changes in S_I as a quantitative trait under recessive genetic models (28).

As a next step in clarifying the conflicting literature and evaluating the effect of *PPARG* P12A on thiazolidinedione response in a large multiethnic sample, we set out to confirm the predictive power of this variant and assess its impact on the lifestyle and pharmacological interventions employed in the Diabetes Prevention Program (DPP) (29). We further examined the five non-redundant SNPs which had shown positive nominal associations with response to troglitazone in the TRIPOD study (28) for a similar effect on troglitazone response in the DPP cohort.

METHODS

The Diabetes Prevention Program

The details of study design and preventive interventions have been described elsewhere (29–31). The DPP was a 27-center randomized clinical trial that examined whether a lifestyle intervention directed at modifying risk factors for type 2 diabetes (overweight, and sedentary lifestyle), or metformin would prevent or delay the development of diabetes in persons at high risk. The DPP enrolled 3,234 nondiabetic persons with IGT and elevated fasting glucose and randomized them to placebo, metformin 850 mg twice daily, or a lifestyle intervention program; a fourth arm of 585 subjects assigned to treatment with troglitazone 400 mg daily was stopped two years after the trial commenced because of hepatotoxicity (30). The principal endpoint was the development of diabetes, confirmed on a second test using ADA criteria. The lifestyle and metformin interventions reduced the incidence of diabetes in high-risk individuals by 58% (95% CI 48–66) and 31% (95% CI 17–43) respectively versus placebo (29). Diabetes incidence rates were 11.0, 7.8, and 4.8 per 100 person-years in the placebo, metformin, and lifestyle groups respectively; treatment effects were consistent across sex and self-reported ethnicity, and diabetes incidence did not differ across ethnic groups (29). When analyses were restricted to the mean 0.9-year period of active troglitazone treatment, diabetes incidence rates were 12.0, 6.7, 5.1 and 3.0 per 100 person-years in the placebo, metformin, lifestyle and troglitazone groups respectively ($P<0.001$, troglitazone versus placebo) (32).

Participants

The 3,548 participants included in this study (92.9% of all DPP participants: 2,994 who completed the trial in their originally assigned treatment groups, plus 554 originally randomized to troglitazone) provided informed consent specific to genetic investigation. The study was approved by the relevant Institutional Review Boards at the participating sites. Of the participants in this study, 56.4% were Caucasian, 20.2% were African American, 16.8% were Hispanic, 4.3% were Asian American and 2.4% were American Indian by self report. The participants' mean age was 51 years and mean body mass index (BMI) was 34.0 kg/m^2. Subjects had semiannual measurements of fasting glucose and glycated hemoglobin, and an annual 75-g oral glucose tolerance test (OGTT); given the early termination of the troglitazone arm, one-year data were available in only 340 of the participants randomly assigned to troglitazone.

PPARG SNP selection

In addition to P12A (rs1801282), we also genotyped five of the eight SNPs reported by Wolford *et al*. to have positive nominal associations with response to troglitazone (28). The TRIPOD investigators found that, of those eight SNPs (rs13073869, rs880663, rs4135263, rs1152003, rs6806708, rs13065455, rs13088205 and rs13088214), the first two and the last three were in perfect linkage disequilibrium with each other, respectively (r^2=1.0); we therefore selected a non-redundant set of five SNPs for our analyses (rs880663, rs4135263 rs1152003, rs6806708 and rs13065455). We confirmed that these SNPs were indeed non-redundant in our five ethnic groups: with the exception of rs6806708 and rs13065455, which were in near-perfect linkage disequilibrium both in the original publication and in our samples (r^2=0.9–1.0), the other SNPs had pairwise r^2 ranging from 0.0–0.2 in Caucasians to 0.1–0.4 in American Indians.

Genotyping

DNA was extracted from peripheral blood leukocytes through conventional procedures and quantitated by picogreen analysis (Molecular Probes). Genotyping of *PPARG* P12A was performed in the forward and reverse directions by allele-specific primer extension of single-plex amplified products, with detection by matrix-assisted laser desorption ionization-time of flight mass spectroscopy on a Sequenom platform as previously described (33); the five other *PPARG* SNPs were genotyped in the same manner but with single-direction primers only. Our genotyping success rate was 99.8% and there were no discordant genotypes on forward and reverse primer extension. The allele frequencies of all six SNPs in each of the five ethnic groups were in Hardy-Weinberg equilibrium (P>0.01).

Quantitative traits

Data from the baseline and one-year OGTTs were used to calculate measures of insulin secretion and insulin sensitivity, which were expressed using glucose and insulin measured in conventional units (milligrams per deciliter and microunits per milliliter, respectively) as previously described (34). The insulinogenic index (35) was calculated as [(insulin at 30 min) − (insulin at 0 min)]/[(glucose at 30 min) − (glucose at 0 min)]). The insulin sensitivity index (ISI, reciprocal of insulin resistance by the homeostasis model assessment (36)) was calculated as 22.5/[fasting insulin × (fasting glucose/18.01)]. In addition, we examined fasting glucose and 2-hour OGTT glucose at baseline and one year.

Statistical analysis

Time to onset of diabetes was the primary endpoint. Because the previous literature consistently reports a recessive model of risk transmission for proline carriers at *PPARG* P12A, P/A and A/A individuals were grouped into one genotypic category (A/X). We examined Cox regression models with genotype, intervention and their interactions as the independent variables predicting time to diabetes. These models were also examined with baseline BMI, waist circumference, age, gender and self-reported ethnicity as covariates. Analyses were repeated in the subset of ethnic groups that had comparable allele frequencies (Caucasians, Hispanics and Asian Americans), and in Caucasians only; whether we restricted our analysis to these subgroups or tested for a genotype × ethnicity interaction, we detected no significant effect of self-reported ethnicity in any of our analyses.

For the quantitative trait comparisons, we first obtained baseline measures in the entire cohort according to genotype at *PPARG* P12A. Differences between means in the two genotypic groups (P/P and A/X) were tested using t tests. For the one-year measurements, a general linear model was examined with and without 3-way interactions (treatment group, genotype, and baseline value of each trait). Least square means were adjusted for baseline values; two-sided

nominal *P* values are reported. The SAS analysis system version 8.2 was used for all analyses (SAS Institute, Inc., Cary, NC).

In order to determine the potential effects of genotype on responsiveness to troglitazone, we calculated the ISI in the 340 DPP participants who completed one year of troglitazone treatment at baseline and one year. In accordance with the previous classification (28), we divided this group into tertiles of change in ISI (one-year ISI minus baseline ISI) and assigned the top two tertiles as "responders" and the bottom tertile as "non-responders"; we then examined allelic frequency differences between the two groups by chi square analysis. In addition, we compared change in ISI as a quantitative trait according to genotype at all five loci by means of the non-parametric Kruskal-Wallis test; if nominally significant differences were found, pairwise comparisons between genotypic groups were performed with a Wilcoxon test, with *P* values adjusted by Holm's procedure as previously described (37). We also compared one-year ISI as a quantitative trait according to genotypic group at all five loci, adjusted for baseline ISI, under the additive and recessive models. Finally, in order to control for allele frequency differences among populations, we repeated these analyses in the largest group (Caucasians) only.

RESULTS

Allele frequency distribution

For *PPARG* P12A, the frequency of the minor alanine allele in DPP US Caucasians (0.10) was comparable to that previously reported in other Caucasian populations (4,6,8,16). We found significant differences in minor allele frequencies in African Americans (0.02) and American Indians (0.19) when compared to Caucasians; therefore, analyses for incidence of diabetes were performed both with and without these two ethnic groups.

At *PPARG* P12A, genotypic frequencies were equally distributed among the four treatment arms and two gender groups. We found no significant differences in baseline age or BMI, but P/P homozygotes appeared to have a smaller waist circumference (Table 1).

Incidence of diabetes

Consistent with previous cross-sectional case/control results, the DPP showed that individuals who were homozygous for the proline allele appeared to progress more rapidly from IGT to diabetes than alanine carriers (HR 1.24, 95% CI 0.99–1.57, $P=0.07$). We found no interaction between genotype and intervention (*P* value for the genotype × metformin interaction, 0.89; *P* value for the genotype × lifestyle interaction, 0.61). Hazard ratios were similar across all treatment arms (Fig. 1). In the placebo group, the hazard ratio was slightly higher but had wider 95% confidence intervals (HR 1.28, 95% CI 0.90–1.82, $P=0.17$). When the sample was restricted to the Caucasian group only, the overall hazard ratio for all treatment groups combined was statistically indistinguishable, but again with wide 95% confidence intervals possibly due to the smaller sample size (HR 1.18, 95% CI 0.89–1.57, $P=0.24$).

When baseline BMI was added to the model, we noted a nominally significant genotype × BMI interaction ($P=0.03$), such that alanine carriers were more susceptible to the deleterious effect of BMI on diabetes incidence than proline homozygotes (Fig. 2). Addition of the BMI interaction term to the model did not significantly alter the overall effect of genotype. Similar effects were noted for waist circumference (which is highly correlated with BMI in this cohort): there was a nominally significant interaction between genotype and waist circumference ($P=0.002$), and in a model adjusting for baseline waist circumference P/P homozygotes were more likely to progress to diabetes than alanine carriers (HR 1.27, 95% CI 1.01–1.60, $P=0.04$).

Quantitative traits

At baseline, proline homozygotes and alanine carriers had indistinguishable indices of insulin sensitivity and insulin secretion (Table 1). At one year, the lifestyle intervention, metformin and troglitazone all led to significant improvements in insulin sensitivity, as previously reported (32,34); however, there were no significant differences in the magnitude of these improvements by genotype at *PPARG* P12A (Table 2). Examination of fasting and 2-hour glucose levels after OGTT both at baseline and at one year did not reveal significant differences between proline homozygotes and alanine carriers across all treatment groups (Table 1 and data not shown).

Response to troglitazone

In addition to *PPARG* P12A, we examined the five *PPARG* SNPs rs880663, rs4135263 rs1152003, rs6806708 and rs13065455 for association with response to troglitazone. The median (25^{th} –75^{th} percentile) ISI (expressed in $[(\mu U/ml) \times (mmol/L)]^{-1}$) for participants randomly assigned to troglitazone treatment at baseline was 0.163 (0.119–0.232). After one year of troglitazone treatment, participants in the bottom tertile of change in ISI ("non-responders") did not show any improvement in ISI (one-year ISI minus baseline ISI, −0.070 ± 0.088, mean ± SD), whereas "responders" in the middle and upper tertiles did (one-year ISI minus baseline ISI, +0.047 ± 0.028 and +0.252 ± 0.180, respectively). There were no significant allele frequency differences at any of the five loci between troglitazone "responders" and troglitazone "non-responders" after one year (Table 3).

When we analyzed change in ISI at one year as a continuous trait, we noted a nominally significant higher change in ISI in rs880663 heterozygotes when compared to homozygotes for either allele (P=0.02–0.04); no other nominally significant differences were found at any of the four remaining SNPs (Table 3). Similar results were obtained when we compared one-year ISI (adjusted for baseline ISI) across genotypic groups. Furthermore, in contrast with the results of Wolford *et al.* (28), homozygotes for the minor allele at all five SNPs had one-year ISI levels (adjusted for baseline ISI) indistinguishable from major allele carriers (P=0.10–0.84). Adjustment for gender, baseline age, baseline BMI or self-reported ethnicity did not change the results. Analyses restricted to the largest ethnic group (Caucasians only, N=201) did not reveal any statistically significant differences in the response to troglitazone.

DISCUSSION

A limited number of common genetic variants have been consistently associated with type 2 diabetes (22): these include *PPARG* P12A, the E23K polymorphism in the gene encoding the islet ATP-sensitive potassium channel Kir6.2 (*KCNJ11*) and SNP44 in the gene that encodes calpain 10 (*CAPN10*). More recently, a common allele in the *TCF7L2* gene has been convincingly associated with type 2 diabetes, with an estimated allelic relative risk of 1.56 and high statistical significance (38). While these validated associations have been usually tested in case/control samples, few studies have examined them prospectively and/or in regard to their effect on therapeutic interventions.

The DPP is a unique study in which to carry out such analyses. It differs from other diabetes prevention trials (39,40) in that it included both lifestyle and pharmacological interventions; in addition, its multiethnic design reflects the diversity of the US population. Moreover, its large sample size makes it adequate for genetic studies where variants are thought to confer modest risk. An important distinction with other large observational trials (25) is the DPP's interventional design and the exclusive enrollment of individuals with IGT, which suggests the presence of some degree of genetic risk at baseline and may introduce selection bias by

imposing constraints at ascertainment. We have recently validated the association of common variants in *TCF7L2* with development of diabetes in this cohort (33).

Genetic studies in multiethnic cohorts raise the issue of population stratification (41). We have addressed this possible confounder by repeating the analyses in the ethnic groups which have comparable allele frequencies, further restricting the analyses to the largest ethnic group alone, and explicitly testing for a genotype × ethnicity interaction. In addition, we note that in the short interval and high-risk population studied in the DPP there were no significant differences in diabetes incidence across ethnic groups (29); thus, it is unlikely that differences in allele frequencies across populations have confounded our phenotypic results.

In agreement with both the Botnia Prospective Study (25) and the preponderance of the cross-sectional literature (and in contrast with the Finnish Diabetes Prevention Study (24)), we also observed a modest genetic risk conferred by the homozygous P/P genotype at *PPARG* P12A. Although the *P* values we obtained do not quite reach conventional statistical significance, the very high prior probability that *PPARG* P12A increases risk of type 2 diabetes makes us believe that the hazard ratios we have noted here represent a real effect. Possible reasons for its lower magnitude in the DPP include the initial high-risk characteristics of the DPP cohort and the limited 3-year window of the IGT-to-diabetes transition examined during this study. It is also possible that this variant may exert a stronger effect on the transition from normoglycemia to IGT, rather than in the progression from IGT to diabetes.

By detecting a strong genotype × obesity interaction we have been able to clarify some of the heterogeneity found in the literature, where studies conducted in leaner populations tend to report higher odds ratios for risk associated with the P/P genotype (3,5). Our data show that the protective effect of the alanine allele disappears at BMIs above ~35 kg/m^2. Indeed, this might partially explain the apparent lack of a protective effect of the alanine allele in the Finnish Diabetes Prevention Study (24), where A/A homozygotes were more obese than P/P homozygotes at baseline (BMI 33.0 ± 6.3 versus 31.1 ± 4.4 kg/m^2 [mean ± SD], respectively). This interaction of *PPARG* P12A with BMI is also consistent with the increased skeletal muscle glucose uptake seen in lean but not obese (BMI >27 kg/m^2) alanine carriers when compared to P/P homozygotes (42).

Despite the well-documented effect that this missense polymorphism (in a gene that encodes the molecular target for thiazolidinedione medications) has on type 2 diabetes, we have been unable to detect a discernible impact of this variant on quantitative glycemic traits such as fasting glucose, 2-hour glucose after an OGTT, or validated measures of insulin secretion and insulin sensitivity. In addition, both a lifestyle intervention and troglitazone treatment for one year improved insulin sensitivity in proline homozygotes and alanine carriers to a similar degree. Our findings support similar results reported in smaller groups of 131 German subjects with type 2 diabetes treated with pioglitazone for 26 weeks (26) or 93 Hispanic women with a history of gestational diabetes treated with troglitazone for 3 months (27), although the length of exposure to thiazolidinediones was modest for all three studies. If the small non-significant differences we observed between genotypic groups are real, we estimate that at least 3,995 subjects would be needed to have 80% power to detect this difference at an alpha of 0.05, which in turn raises the question of its clinical relevance.

It is possible that other genetic variants at *PPARG* may affect thiazolidinedione response, even though none of them has been convincingly associated with type 2 diabetes. Recently, a comprehensive set of common variants in *PPARG* was genotyped in the TRIPOD group of 93 Hispanic women with a history of gestational diabetes, and examined for their impact on response to troglitazone. Allele frequencies at eight *PPARG* SNPs differed between the 63 responders and 30 non-responders, although the sample was small and the *P* values modest

(28). We have been unable to reproduce these findings of association for five of those SNPs in our larger cohort of 340 subjects. This lack of replication may be due to the differences in duration of troglitazone treatment (three months in TRIPOD versus 1 year in the DPP), differing estimates of insulin sensitivity (S_I from IVGTT versus ISI from OGTT), phenotypic heterogeneity (gestational diabetes versus IGT), ethnic variation or statistical fluctuations: nevertheless, because the 95% confidence intervals between both studies overlap, we cannot exclude that the results are mutually consistent. The nominally significant higher change in ISI at one year in rs880663 heterozygotes in the DPP does not conform with the published data and does not follow a clear genetic model; given the multiple tests performed, this finding likely represents a false positive result. The next logical step will be to test comprehensively all common variation at *PPARG* in the various ethnic groups of the DPP.

In summary, we have confirmed the modest protection from type 2 diabetes conferred by the alanine allele at *PPARG* P12A, we have shown a significant interaction of this variant with BMI and waist circumference, and in examining the largest cohort studied to date we have not detected any significant effect of genotype at *PPARG* P12A in response to troglitazone.

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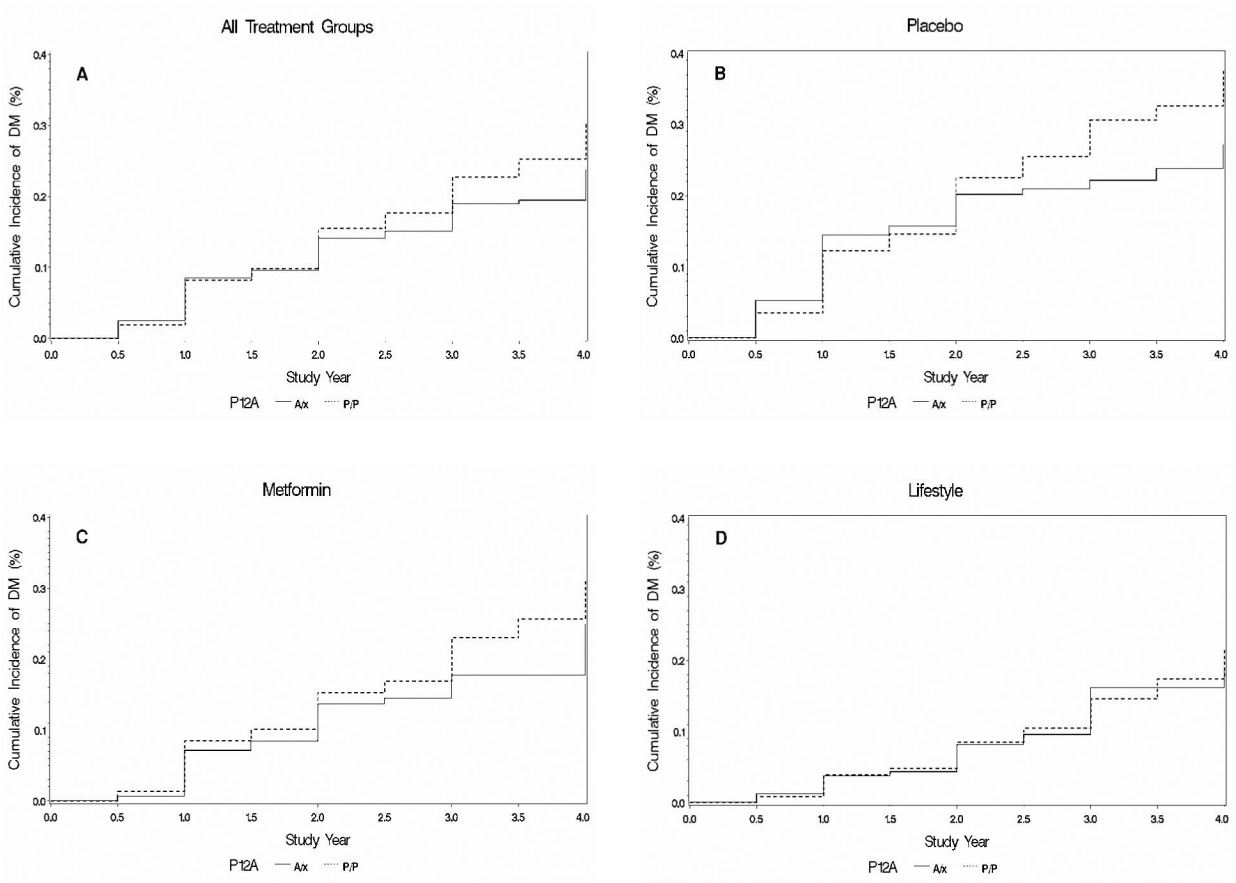

Figure 1.
Incidence of diabetes per treatment arm and genotype at *PPARG* P12A in the Diabetes Prevention Program. A, all arms; B, placebo; C, metformin; D, lifestyle intervention.

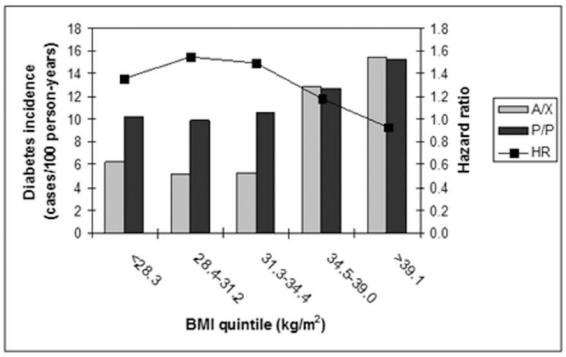

Figure 2.

Interaction of BMI with genotype at *PPARG* P12A on diabetes risk. The bar plot (left axis) shows incidence of diabetes (cases/100 person-years) in the placebo arm by quintile of baseline BMI for either alanine carriers or proline homozygotes at *PPARG* P12A. The line plot (right axis) shows the hazard ratio (HR, P/P versus A/X) in the full DPP cohort by quintile of baseline BMI. The protective effect of alanine seems to disappear at BMI >34.5 kg/m^2.

Table 1

Demographic characteristics and baseline quantitative traits according to genotype at *PPARG* P12A in the Diabetes Prevention Program

Baseline trait	Genotype		*P**
	P/P (n=2,942)	A/X (n=605)	
Treatment			
Placebo	831 (83.1)	169 (16.9)	0.14
Metformin	831 (84.0)	158 (16.0)	
Lifestyle	839 (83.6)	165 (16.4)	
Troglitazone	441 (79.6)	113 (20.4)	
Male	957 (81.7)	214 (18.3)	0.18
Age in years	50.8 ± 10.5	50.9 ± 10.9	0.83
Body mass index (kg/m^2)	34.0 ± 6.7	34.2 ± 6.4	0.41
Waist (cm)	105 ± 14.5	107 ± 14.5	0.002
Glycemic trait			
Ins Index [(μU/ml)/(mg/dl)]	1.24 ± 0.93	1.23 ± 0.90	0.73
ISI $[(\mu U/ml)x(mmol/L)]^{-1}$	0.194 ± 0.130	0.194 ± 0.126	0.93
Fasting glucose (mg/dl)	106.9 ± 8.2	106.7 ± 8.1	0.60
2-hour glucose (mg/dl)	164.8 ± 17.0	164.5 ± 17.3	0.66

Plus-minus values are means ± SD, other values are n (%).

* Based on t-tests for continuous variables and chi-square for categorical variables; traits were log transformed for statistical analyses where appropriate. Ins Index, insulinogenic index; ISI, insulin sensitivity index. One sample failed genotyping.

Table 2

One-year quantitative glycemic traits according to *PPARG* P12A genotypes by treatment arm in the DPP

	Genotype		
Ins Index [(μU/ml)/(mg/dl)]	**P/P**	**A/X**	***P* value**
Placebo	1.21 (1.14–1.27)	1.24 (1.09–1.38)	0.72
Metformin	1.20 (1.14–1.27)	1.16 (1.01–1.31)	0.62
Lifestyle	1.18 (1.12–1.25)	1.29 (1.14–1.43)	0.20
Troglitazone	1.25 (1.13–1.37)	1.10 (0.86–1.33)	0.25
ISI $[(\mu U/ml)x(mmol/L)]^{-1}$	P/P	A/X	*P* value
Placebo	0.190 (0.183–0.198)	0.192 (0.176–0.208)	0.87
Metformin	0.234 (0.225–0.243)	0.234 (0.214–0.254)	0.99
Lifestyle	0.268 (0.257–0.279)	0.292 (0.267–0.318)	0.09
Troglitazone	0.268 (0.248–0.289)	0.289 (0.249–0.328)	0.37

Values are expressed as least-squares means (adjusted for baseline values) with 95% confidence intervals. Ins Index, insulinogenic index; ISI, insulin sensitivity index.

Table 3

Association testing of five *PPARG* SNPs for response to troglitazone at one year in the Diabetes Prevention Program

			Responders vs. Non-responders		Change in ISI at one year [median (range)]				
SNP	**Alleles (M/m)**	**MAF**	**OR (95% CI)**	***P* ***	**M/M**	**M/m**	**m/m**	***P* †**	***P* ‡**
rs880663	T/C	0.29	1.18 (0.83–1.69)	0.36	0.034 (−0.573/ +0.778)	0.062 (−0.286/ +1.069)	0.016 (−0.149/ +0.938)	0.005	TT v CT: 0.02 CT v CC: 0.04 TT v CC: 0.35
rs4135263	T/C	0.17	0.88 (0.58–1.34)	0.55	0.050 (−0.573/ +0.997)	0.039 (−0.333/ +1.069)	0.021 (−0.052/ +0.186)	0.94	
rs1152003	C/G	0.45	1.02 (0.74–1.40)	0.91	0.046 (−0.286/ +0.778)	0.051 (−0.337/ +1.069)	0.028 (−0.573/ +0.938)	0.94	
rs6806708	G/T	0.35	0.96 (0.68–1.33)	0.79	0.043 (−0.337/ +0.659)	0.048 (−0.573/ +0.997)	0.056 (−0.142/ +1.069)	0.86	
rs13065455	C/A	0.34	0.93 (0.67–1.31)	0.69	0.047 (−0.337/ +0.659)	0.047 (−0.573/ +0.997)	0.056 (−0.142/ +1.069)	0.86	

Five non-redundant *PPARG* single nucleotide polymorphisms (SNPs) were tested for association with response to troglitazone in the DPP after one year of treatment. M/m, major allele/minor allele; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval; ISI, insulin sensitivity index; *P* *, *P* value for the allelic association of the minor allele with response to troglitazone, defined as a larger proportion of minor alleles in the upper two tertiles of change in ISI by chi square analysis; *P* †, *P* value (non-parametric Kruskal-Wallis test) of the comparison of change in ISI after one year across all three genotypic groups; *P* ‡, *P* value (Wilcoxon test) of the pairwise comparison between genotypic groups, performed if the initial test was nominally significant and corrected for multiple comparisons by the Holm procedure.