Analysis of the Relationship Between the Pro12Ala Variant in the PPAR-γ2 Gene and the Response Rate to Therapy With Pioglitazone in Patients With Type 2 Diabetes

MATTHIAS BLÜHER, MD[1]
GEORG LÜBBEN, MD[2]
RALF PASCHKE, MD[1]

OBJECTIVE — To investigate the influence of peroxisome proliferator–activated receptor-γ (PPAR-γ) gene variants on the response rate to therapy with the thiazolidinedione (TZD) pioglitazone, because in vitro studies have suggested that genetic variants of the PPAR-γ gene may influence the drug efficacy of TZD.

RESEARCH DESIGN AND METHODS — A total of 131 patients were treated in an open-label, randomized, multicenter study with pioglitazone (45 mg o.d.) during a course of ≥26 weeks. Response to the pioglitazone therapy was defined by either a >20% decrease in fasting plasma glucose or a >15% decrease in HbA_{1c} values after 26 weeks of pioglitazone treatment. We evaluated the association between the PPAR-γ genotype and the response rate to pioglitazone treatment.

RESULTS — The Pro12Ala and the Pro12Pro variants in the PPAR-γ gene are not associated with the response rate to pioglitazone treatment in patients with type 2 diabetes. However, we identified initial fasting plasma glucose level >11.0 mmol/l, HbA_{1c} value >9.0%, BMI >32 kg/m^2, and fasting C-peptide concentrations at baseline >2.5 pmol/l as predominant confounding factors for the responder frequency to pioglitazone treatment.

CONCLUSIONS — The Pro12Ala variant in the PPAR-γ gene does not affect the therapy efficacy of pioglitazone, suggesting that the drug-treatment response is independent from pharmacogenetic effects between PPAR-γ and its ligand pioglitazone. Whether the Ala12Ala genotype plays a role in the response rate to TZD therapy remains to be determined.

Diabetes Care **26:825–831, 2003**

Pharmacogenetics has become increasingly accepted as an important consideration in the therapeutic decision-making process (1). The identification of inherited differences between individuals, which can predict each patient's response to a drug, will therefore be a new tool in development of drugs and clinical application of medications (2). Such genotype drug-response interactions have been described for the efficiency of ACE inhibitor treatment depending on the ACE genotype (3,4); for the cholesterol-lowering effect of pravastatin, depending on the genotype of the cholesterol ester transfer protein (5); and for formoterol-depending on the β_2, adrenoreceptor genotype (6). Moreover, in patients with asthma, it was shown that resistance to leukotriene therapy is dependent on the ALOX5 promoter genotype (7) and that polymorphisms in the promoter and coding region of the 5-HT2A receptor gene cause different therapeutic effects of clozapine (8).

Therefore, heterogeneity at the molecular level provides an important explanation for differential individual drug response and might also explain the nonresponder rate or an unexpectedly high response to drug treatment.

The interaction of thiazolidinedione (TZD) with the peroxisome proliferator–activated receptor-γ (PPAR-γ) represents another model of pharmacogenomics. TZD has been shown to decrease plasma glucose concentrations in patients with type 2 diabetes (9–11). Clinical studies showed that ~10–25% of the patients treated with TZD did not achieve a 15% reduction in fasting plasma glucose or did not convert from impaired glucose tolerance to normal glucose tolerance (12). However, the molecular reasons for the different responses to TZD therapy have not been determined, and differences in the PPAR-γ genotype may modify the response to TZD treatment. Therefore, we investigated whether polymorphisms in the PPAR-γ gene are a cause for different responses or therapy resistance in 131 patients with type 2 diabetes treated for at least 26 weeks with the TZD pioglitazone. We compared the frequencies of the previously described Pro115Gln (13), Pro12Ala (14), Val290Met, Pro467Leu (15), and one silent polymorphism (16) between a group of patients responsive to the therapy with pioglitazone, defined by either a >20% decrease in fasting plasma glucose concentrations or a >15% de-

From the [1]Medical Department, Faculty of Medicine, University of Leipzig, Leipzig, Germany; and [2]Takeda Pharma, Aachen, Germany.

Address correspondence and reprint requests to Prof. Dr. med. R. Paschke, University of Leipzig, III. Medical Department, Philipp-Rosenthal-Straβe 27, D-04103 Leipzig, Germany. E-mail: pasr@medizin3.uni-leipzig.de.

Received for publication 22 May 2002 and accepted in revised form 15 November 2002.

Abbreviations: DGGE, denaturing gradient gel electrophoresis; PPAR-γ, peroxisome proliferator–activated receptor-γ, TZD, thiazolidinedione.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

crease in HbA_{1c} values after 26 weeks of TZD treatment, and a group of patients resistant to the therapy. Moreover, with the aim of defining the best responders to TZD therapy on the molecular level, we evaluated the association between the PPAR-γ genotype and a high response to pioglitazone treatment in a subgroup of individuals who achieved a >40% decrease in fasting plasma glucose or HbA_{1c} values with pioglitazone treatment.

RESEARCH DESIGN AND METHODS

Subjects and study design

A total of 268 patients were treated in an open-label, randomized, multicenter study with either pioglitazone (45 mg o.d.) or acarbose (50–100 mg t.i.d.) in a parallel group design with two treatment arms during a course of at least 26 weeks. A total of 131 patients on pioglitazone therapy were included in the analysis. Written consent was obtained from each subject after the nature of the study and the procedures were explained prior to participation in the study. After a 1-week run-in period (only diet treatment) patients were investigated at baseline and at 4, 8, 12, 16, 20, and 26 weeks after enrollment in the study. Patients with type 2 diabetes with HbA_{1c} ≥7.5 and ≤11.5% and fasting serum glucose ≥7.8 and ≤14.0 mmol/l were chosen according to the following inclusion criteria: *1*) men or women aged ≥35 or ≤75 years; *2*) BMI ≥25 or ≤35 kg/m^2; *3*) written informed consent given; and *4*) women who were postmenopausal, hysterectomized, surgically sterilized, or using appropriate contraceptive methods.

In addition, patients with type 1 diabetes, current exposure to antidiabetic medication or necessary concomitant therapy with other antidiabetic drugs, any history of ketoacidosis, any disease with malabsorption, acute or chronic pancreatitis, chronic gastrointestinal diseases with significant digestion and resorption disturbances, conditions that may be aggravated by increased gas in the intestine, any history of unstable angina pectoris or any other unstable heart disease, heart failure of New York Heart Association (NYHA) stage II-IV, history of any malignant disease within the last 10 years, any infection with HIV, significant anemia of any etiology or any other clinically relevant hematological disease, any serious preexisting impairment of liver, kidney, or bone marrow function, >3 years of phenacetin use, donation of blood during the study, any history of alcohol or drug abuse, pregnancy/lactation, as well as use of medications with a laxative or constipating effect or affecting gastrointestinal motility and/or absorption were excluded from the study. From the initially enrolled 532 patients who initially met the inclusion criteria, 264 patients were withdrawn from the study because at least one of the exclusion/withdrawal criteria was fulfilled at some point during the study. A total of 76 patients were withdrawn before entering the treatment arms after the run-in period at the baseline visit. From the remaining 188 subjects, 97 patients were withdrawn from the pioglitazone study arm and 91 subjects were withdrawn from the acarbose arm.

Responders and nonresponders to the therapy with TZD were defined as follows: responders, >15% decrease in HbA_{1c} levels and/or >20% decrease in fasting blood glucose levels after 12 or 26 weeks of pioglitazone treatment as compared with baseline; and nonresponders, HbA_{1c} >11.5% or fasting blood glucose >14.0 mmol/l during the study course and <15% decrease in HbA_{1c} levels and/or <20% decrease in fasting blood glucose levels after 12 or 26 weeks of pioglitazone treatment as compared with baseline. In addition, the occurrence of clinical symptoms of diabetes after beginning pioglitazone therapy and unexplained excretion of ketones in the urine was considered nonresponse.

Clinical laboratory

Blood samples were collected at all visits after an overnight fast to determine fasting blood glucose, HbA_{1c}, fasting C-peptide, fasting insulin, total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, triglycerides, and routine chemistry and hematology standard laboratory parameters. Fasting blood glucose, total cholesterol, and triglyceride concentrations were measured with enzymatic assays. C-peptide and insulin plasma levels were measured using immunoassays, the HbA_{1c} values were determined with a cation exchange/high-performance liquid chromatography method (reference values 3.4–6.0%), and the HDL, LDL, and VLDL cholesterol concentrations were measured using lipoprotein electrophoresis. Albuminuria was evaluated at baseline and 26 weeks after initiation of treatment. Analysis of all blood samples was performed using standard methods by a central laboratory (Dr. W. März, University of Freiburg, Germany).

Preparation of genomic DNA and screening for the PPAR-γ mutations

Genomic DNA was isolated from human leukocyte nuclei isolated from whole blood by proteinase K digestion using QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA). PCR amplification of the segments with the PPAR-γ mutations was performed in a total volume of 50 μl containing PCR buffer (10× Optiperform Buffer III: 500 mmol/l potassium hydroxide, pH 9.2 at 25°C; 160 mmol/l $(NH_4)_2SO_4$; 0.1% Tween 20; and 2 mmol/l $MgCl_2$), 5 μl cDNA with 1 unit of CombiPool DNA Polymerase Mix in vitro, 0.5 mmol/l dNTPs, and 1 μmol/l sense and antisense primers. The PCR conditions for the Pro12Ala mutation, the Pro467Leu mutation, and the silent polymorphism CAC478CAT were as follows: denaturation at 94°C for 3 min, followed by 34 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, extension at 72°C, and a final extension at 72°C for 7 min. For the Pro115Gln and the Val290Met mutation, PCR was performed as described above except for the number of cycles (36 cycles) and the annealing temperature (54°C). The selection of all primers was based on the human genomic PPAR-γ2 sequence. All primer pairs span introns. The following primer pairs were selected: Pro12Ala: forward primer: 5′-GGC TCC TAA TAG GAC AGT GCC-3′, reverse primer: 5′-TAC CCT TAC ATA AAT GCC CCC-3′; Pro115Gln: forward primer: 5′-TTG CCC TGT TGC CTT TTT AG-3′, reverse primer: 5-CAC CTA AAA AAG GGG TTC TGC-3′; Val290Met: forward primer: 5′-AGT CAT CCA CGT TTT CCC TG -3′, reverse primer: 5′-TCC AAA ATT CTT TTG GCC AC -3′; and Pro467Leu and CAC478CAT: forward primer: 5-TTT TTT GAC TGA ACC CCC TG-3′, reverse primer: 5′-TGG AAG AAG GGA AAT GTT GG-3. As a screening method for the detection of the PPAR-γ2 gene mutations, denaturing gradient gel electrophoresis (DGGE) was used. For DGGE, one GC-clamped primer of each described primer pair was used. For mutation detection by DGGE, heteroduplices between wild-type and mutant alleles were generated and the optimal

Table 1—*Clinical characterization of the study group according to the PPAR-γ genotype in position 12*

	Study group	Pro12Pro	Pro12Ala	Ala12Ala
Patients (men/women)	131 (71/60)	110 (57/53)	16 (10/6)	5 (4/1)
Age (years)	60.7 ± 9.3	61.1 ± 9.7	57.9 ± 5.3	61 ± 6.0
Duration of diabetes (years)	6.51 ± 2.9	6.9 ± 5.1	4.4 ± 2.4	5.8 ± 2.2
BMI				
Baseline	31.0 ± 3.3	30.9 ± 3.0	31.4 ± 3.1	33.4 ± 2.2
6 months after treatment	31.4 ± 3.6	31.4 ± 3.43	31.2 ± 3.1	34.8 ± 1.8
Fasting plasma glucose (mmol/l)				
Baseline	12.7 ± 0.8	12.6 ± 0.9	13.4 ± 0.3	12.0 ± 0.8
6 months after treatment	10.1 ± 1.3*	9.6 ± 1.3*	10.2 ± 1.2*	9.9 ± 1.1*
HbA_{1c} (%)				
Baseline	8.94 ± 1.2	8.92 ± 1.2	9.15 ± 1.1	8.84 ± 0.9
6 months after treatment	7.8 ± 1.5*	7.7 ± 1.0*	8.18 ± 1.2*	7.34 ± 1.4*
C-peptide (pmol/l)				
Baseline	3.37 ± 1.17	3.42 ± 1.8	3.13 ± 1.1	3.18 ± 1.9
6 months after treatment	2.58 ± 1.0*	2.59 ± 1.49*	2.3 ± 1.1*	2.68 ± 0.7*

Data are means ± SD. *Significant differences between baseline (T0) and 6 months after pioglitazone treatment ($P < 0.05$).

DGGE conditions were established for each DNA. The determined optimal DGGE conditions were as follows: Pro12Ala, Pro115Gln, Pro467Leu, 6% polyacrylamide gel containing a linear gradient of 20–40%, 3-h running time; and Val290Met, 6% polyacrylamide gel containing a linear gradient of 20–60%, 6 h running time. Confirmation of the detected Pro12Ala and Ala12Ala variants and the detection of the silent polymorphism CAC478CAT was performed using direct sequencing (ABI Prism Sequencer System).

Statistical analysis

Data are shown as the mean ± SD. All calculations and statistics were performed using SPSS for Windows (release 7.5; SPSS, Chicago, IL). The differences between the groups were tested using one-way ANOVA. If the *P* value was <0.05, the groups were compared using the appropriate test (Student's *t* test for unpaired samples or χ^2 test). A *P* value <0.05 was considered significant. Correlations between variables were tested using Spearman's correlation test. A correlation coefficient (*r*) of $P < 0.05$ was considered significant. To obtain the odds ratios for a possible specific influence of the PPAR-γ genotype on BMI, C-peptide, and plasma lipid levels, multivariate logistic regression analysis was performed.

RESULTS — The data of 268 patients who completed the entire study and 188 early withdrawn patients were analyzed. A total of 131 patients (71 men, 60 women) were treated with 45 mg o.d. pioglitazone and 137 patients were treated with 50–100 mg t.i.d. acarbose. Only data from pioglitazone-treated patients were analyzed for assessment of the possible correlation between the efficacy of pioglitazone treatment and the PPAR-γ genotype. The clinical and biochemical characteristics of the 131 patients who fulfilled the complete course of pioglitazone treatment are summarized in Table 1. The frequencies of the Pro12Ala and Ala12Ala variant of the PPAR-γ gene were not significantly different between the acarbose- and pioglitazone-treated groups. Furthermore, the PPAR-γ allele and genotype distribution was not different between the acarbose- and pioglitazone-treated early-withdrawn patients and was not different from the distribution in the 268 patients who completed the study. From the remaining 188 subjects, 97 patients were withdrawn from the pioglitazone study arm and 91 subjects were withdrawn from the acarbose arm. Of the 97 withdrawn patients from the pioglitazone study, 88 patients were withdrawn due to HbA_{1c} values >11.5% or fasting plasma glucose >14.0 mmol/l. The PPAR-γ genotype distribution for these patients was as follows: Pro12Pro 84.9% (75 patients), Pro12Ala 11.7% (10 patients), and Ala12Ala 3.4% (3 patients). The distribution of the different PPAR-γ genotypes was not different from the PPAR-γ genotype distribution in the 131 patients who completed the study. In the 131 patients who completed the study, the allelic frequency of the Pro12Ala polymorphism was 12.2% (10 men, 6 women); the frequency of the Ala12Ala variant was 3.8% (four men, one woman) in the pioglitazone treatment group, and in the acarbose-treated group, the frequency was 12.8% for the Pro12Ala variant and 2.7% for the Ala12Ala variant. Therefore, we excluded a PPAR-γ genotype selection bias in the pioglitazone treatment group. Only two patients (1.5%) with the Pro12Pro genotype were detected with an additional silent polymorphism C478T in exon 6. The previously described Pro115Gln (13), Pro467Leu, and Val290Met mutations (15) were not detected in any patient.

In the entire study population, the response rate to pioglitazone treatment, as defined by a >20% decrease in fasting blood glucose was achieved in 51.9% of the patients after 3 or 6 months of treatment (Fig. 1). The rate of nonresponders using the criterion >20% decrease in fasting blood glucose was 48.1% after 3 or 6 months of treatment. Using the criterion >15% decrease in HbA_{1c} value, the rate of responders was higher in the entire study population (61.1% after 3- or 6-month pioglitazone treatment) compared with the fasting blood glucose criterion (Fig. 1). Using criteria from previous studies (9,12,17) for the effect of pioglitazone treatment in our population, i.e., >10% decrease in fasting plasma glucose or 0.5–1% decrease in HbA_{1c}, the response rate would be 75.5% for the fasting plasma glucose concentrations and 87.9% for the HbA_{1c} values. Using decrease in HbA_{1c} <7% or decrease in fasting plasma glucose <6.0 mmol/l, as well as >10, 20, and 30% decrease in HbA_{1c} or fasting plasma glucose as criteria to define the response rate, there was no association between the Pro12Ala and Pro12Pro variants in the PPAR-γ gene and the response to pioglitazone treatment. There were no significant differences between the responder frequency to pioglitazone treatment between the Pro12Pro and the Pro12Ala, variant. However, because only five carriers of the Ala12Ala variant were detected, the statistical power in this study is not sufficient to detect significant differences in the response rate as compared with the other PPAR-γ genotypes in position 12.

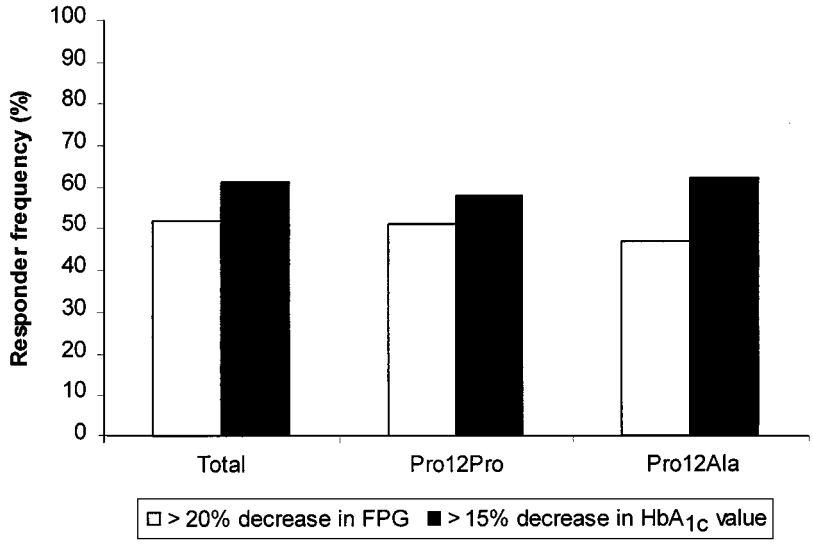

Figure 1—*Frequency of responders to pioglitazone treatment in function of the PPAR-γ2 genotypes Pro12Pro and Pro12Ala. Response was defined as either a >20% decrease in fasting plasma glucose (FPG) (□) or a >15% decrease in HbA_{1c} values (■) after 3 or 6 months of pioglitazone treatment as compared with the baseline values.*

The plasma lipid profile was not significantly different between carriers of the different PPAR-γ variants in position 12 at baseline (Table 2). However, after 6 months of pioglitazone treatment, plasma triglyceride concentrations were significantly decreased in patients with the Pro12Pro and Pro12Ala genotype ($P < 0.05$) and HDL cholesterol concentrations were significantly increased in the four men who were Ala12Ala carriers ($P < 0.05$) (Table 2). At baseline, plasma C-peptide concentrations were not significantly different between the different PPAR-γ genotypes (Table 1). After 6 months of pioglitazone treatment, C-peptide levels decreased in 81.5% of the patients ($P < 0.05$). However, a different response in function of the PPAR-γ genotype was not detectable using significantly decreased C-peptide concentrations as definition of the responders.

In addition, a multivariate regression analysis was performed to evaluate a potential relationship between the PPAR-γ genotype in position 12 and the response rate to pioglitazone treatment using the different criteria, BMI, C-peptide, total, HDL, LDL, and VLDL cholesterol levels, and serum triglyceride concentration. However, only the Ala12Ala variant was significantly related to BMI (Table 3). There was no evidence that the PPAR-γ genotype in position 12 is associated with the response rate to pioglitazone treatment or plasma lipid parameters (Table 3).

To define the major confounding factors for the individual response to pioglitazone treatment in this study, we performed a multivariate regression analysis for the influence of several clinical and biochemical parameters on the response rate, defined by a >15% decrease in HbA_{1c} values after 6 months of pioglitazone therapy. In this analysis, we identified an initial fasting plasma glucose level >11.0 mmol/l, HbA_{1c} value >9.0%, BMI >35 kg/m^2, and fasting C-peptide concentrations at baseline >2.5 pmol/l as predominant confounding factors for the responder frequency to pioglitazone treatment (Table 4). We confirmed in the multivariate regression analysis that the PPAR-γ genotype in position 12 has no significant effect on the responder rate to pioglitazone treatment (Table 4).

To exclude the influence of the detected confounding factors for the responder rate to pioglitazone treatment in our study population, we analyzed subgroups, matched for different baseline fasting plasma glucose concentration ranges, baseline HbA_{1c} value ranges, age ranges, duration of diabetes, and BMI ranges. There were no significant differences in the frequency of responders to pioglitazone treatment between the Pro12Pro and the Pro12Ala variants, depending on the baseline fasting plasma glucose concentrations, the baseline HbA_{1c} value, BMI, and age. However, duration of diabetes was a significant factor influencing the responder rate in carriers of the Pro12Ala variant. No carrier of the Pro12Ala variant with a duration of diabetes of >2 years responded to pioglitazone therapy.

CONCLUSIONS— PPAR-γ was identified as the target for TZD (18). Moreover, different genetic variants of the PPAR-γ gene were shown to cause a dif-

Table 2—*Plasma lipid parameters at the baseline (T0) and 6 months after treatment with pioglitazone 45 mg per day for the different genotypes*

Lipid parameter	Pro12Pro	Pro12Ala	Ala12Ala	*P*
Total cholesterol (mg/dl)				
T0	226 ± 42	215 ± 28	221 ± 16	NS
6 months after treatment	212 ± 51	219 ± 32	198 ± 29	NS
HDL cholesterol (mg/dl)				
Men at T0	33.4 ± 4.5	35.2 ± 3.9	36.1 ± 8.2	NS
Men at 6 months after treatment	37.2 ± 3.7	38.3 ± 2.6	39.3 ± 5.9*	NS
Women at T0	46.7 ± 6.9	44.5 ± 5.4	42.1 ± 6.3	NS
Women 6 months after treatment	48.2 ± 7.2	45.1 ± 6.2	45.5 ± 4.3	NS
LDL cholesterol (mg/dl)				
T0	136 ± 37	130 ± 23	132 ± 40	NS
6 months after treatment	130 ± 42	133 ± 39	127 ± 25	NS
VLDL cholesterol (mg/dl)				
T0	50.1 ± 15.1	48.8 ± 12.1	50.2 ± 10.7	NS
6 months after treatment	38.5 ± 9.1	32.4 ± 8.7	33.1 ± 11.5	NS
Triglycerides (mg/dl)				
T0	261 ± 38	309 ± 24	269 ± 28	NS
6 months after treatment	158 ± 38*	162 ± 39*	205 ± 63	NS

Data are means ± SD. *Significant differences between baseline (T0) and 6 months after pioglitazone treatment ($P < 0.05$).

Table 3—*Logistic regression analysis for the association between PPAR-γ genotype in position 12 and clinical and biochemical parameters*

Parameter	Odds ratio (*P* value)		
	Pro12Pro	Pro12Ala	Ala12Ala
Nonresponder rate (using criteria >20% decrease in fasting plasma glucose)	0.45 (0.67)	0.78 (0.31)	0.61 (0.76)
Nonresponder rate (using criteria >15% decrease in HbA_{1c} value)	0.66 (0.23)	0.42 (0.2)	0.48 (0.61)
BMI	0.28 (0.43)	0.26 (0.5)	2.43 (0.02)*
C-peptide	0.77 (0.5)	0.81 (0.8)	0.91 (0.07)
Total cholesterol	1.09 (0.62)	0.87 (0.79)	0.61 (0.5)
LDL cholesterol	0.94 (0.33)	1.03 (0.7)	0.77 (0.71)
HDL cholesterol	0.51 (0.13)	0.48 (0.3)	0.40 (0.69)
VLDL cholesterol	0.23 (0.6)	0.19 (0.4)	0.19 (0.6)
Triglycerides	0.52 (0.3)	0.51 (0.7)	0.52 (0.6)

*Significant odds ratio ($P < 0.05$).

ferent drug efficacy in vitro (19). Therefore, variants in the PPAR-γ gene could cause differences in the efficiency of TZD therapy in the clinical application and might provide a molecular definition of either very good responders or nonresponders to TZD treatment. Therefore, we investigated whether known variants in the PPAR-γ gene are associated with either resistance or high response to the therapy with the TZD pioglitazone.

The most common variant in the PPAR-γ gene, the Pro12Ala variant, occurs with a frequency of ~12–15% (14,16,20–23). Other mutations are very rare (13,15,24) and therefore unlikely to be a major cause for therapy modification by TZD. Because of its prevalence, the Pro12Ala variant is the first candidate for the possible relationship between PPAR-γ genotype and response to TZD treatment. In our study of patients with type 2 diabetes, the frequency of the Pro12Ala and Ala12Ala polymorphism in the PPAR-γ2 gene was similar to that reported in previous studies investigating obese patients with type 2 diabetes (14,16,20–22,25). The genotype frequencies did not deviate from those expected on the basis of the Hardy-Weinberg equilibrium, although subjects with the Pro12Ala and Ala12Ala genotype were somewhat more frequent than expected based on the allele frequency ($P = 0.08$). A prediction of the expected phenotype of the Pro12Ala carriers is difficult, because the Pro12Ala polymorphism has been variably associated with either increased (21,26), decreased (16), or unchanged (20,22,27) BMI and improved insulin sensitivity (16,23,25). Moreover, it was recently suggested that the Pro12Ala variant may influence the susceptibility for obesity (27) and that this variant might be a genetic marker indicating the risk of obesity in children persisting into adolescence (28). In contrast to Japanese men (29) but in accordance with results from previous studies among Caucasians (21,30), the homozygous Ala12 variant was associated with increased BMI. In accordance with the normal weight or slightly overweight subjects (16,25), the overweight Ala12Ala carriers in our study had C-peptide concentrations that were indistinguishable from the other genotypes in position 12. Because only five carriers of the Ala12Ala were detected in our study, the statistical power to identify significant differences between the investigated parameters was far below 80%. Therefore, we were not able to establish a statistically significant association of this genotype with the response rate to TZD therapy, although this variant may be important in the response rate to pioglitazone therapy. More than 1,000 patients would be needed for detection of a possible association between the Ala12Ala variant and the response rate to pioglitazone treatment according to calculation based on allele frequency, effect size, and population variance, as suggested in a recent meta-analyis of the Pro12Ala variant in the PPAR-γ2 gene (31).

We did not find any subject with the recently reported Pro115Gln (13), Pro467Leu, or Val290Met mutations (15). Therefore, no conclusion regarding a potential effect of these mutations on the response rate to TZD therapy can be drawn from our data. A more frequent silent polymorphism CAC478CAT in exon 6 of the PPAR-γ2 gene (16) was also not associated with the therapeutic effect of pioglitazone in our study group.

To define the responders to pioglitazone therapy, we used a decrease of >20% in fasting blood glucose concentration as compared with baseline or a >15% decrease in HbA_{1c} values after 3 or 6 months as compared with the baseline of pioglitazone therapy. The definition of response rate (>15% decrease in HbA_{1c} values and/or >20% decrease in fasting blood glucose) was chosen according to

Table 4—*Multivariate regression analysis for the influence of clinical and biochemical factors on the response rate to thiazolidinedione treatment using the criterion >15% decrease of HbA_{1c} values 6 months after pioglitazone therapy*

Parameter	Regression coefficient (*r*)	Level of significance (*P*)
Age	0.36	NS
BMI >33 kg/m^2	0.77*	<0.05
Duration of diabetes	0.38	NS
Fasting plasma glucose >14 mmol/l	0.58*	<0.05
Fasting plasma glucose 11.0–14 mmol/l	0.75*	<0.001
Fasting plasma glucose <11 mmol/l	0.32	NS
HbA_{1c} >9.0%	0.82*	<0.001
C-peptide >2.5 pmol/l	0.64*	<0.05
Pro12Pro PPAR-γ genotype	0.29	NS
Pro12Ala PPAR-γ genotype	0.25	NS
Ala12Ala PPAR-γ genotype	0.19	NS

*Significant regression coefficients ($P < 0.05$).

calculations that a significant decrease in fasting blood glucose and HbA_{1c} must be at least >2 SD of the variance of the method and at least >2 SD of the day-to-day differences in glucose and HbA_{1c} measurements. Using these criteria, the nonresponder rate in our study population was much higher (48.1%) as compared with the reported ~20–30% nonresponder frequency in previous studies in patients with type 2 diabetes or impaired glucose tolerance (11,12, 17,32,33). In these studies, the criteria for the nonresponse rate was defined as decreasing HbA_{1c} by 0.5–1% compared with the initial glycemic status and/or decreasing fasting plasma glucose by ≥10%. However, using these criteria would result in an overestimation of the response rate in our study group, because a subgroup of patients with not statistically significant changes in higher initial HbA_{1c} values and fasting plasma glucose concentrations after pioglitazone treatment would be considered responders. This aspect is supported by our finding that the nonresponder rate is significantly decreased in patients with higher initial fasting blood glucose, higher initial C-peptide levels, and insufficient glycemic control (HbA_{1c} >9.0% at baseline). However, the therapeutic target, fasting plasma glucose concentration <6.0 mmol/l and HbA_{1c} value <6.5% was only achieved by <30% of the treated patients, because this threshold is also dependent on other confounding factors.

To test the hypothesis that a different definition of the response rate might reveal a potential relationship between the Pro12Ala and Pro12Pro variant and the response rate to pioglitazone therapy, we analyzed our data using the following definitions of response to pioglitazone therapy: decreasing fasting plasma glucose and/or HbA_{1c} value by >10, 20, or 30% as well as decreasing HbA_{1c} value to <7% or decreasing fasting plasma glucose to <6.0 mmol/l to meet clinically relevant target values. Independent of the definition of response rate to pioglitazone treatment, there was no association between the Pro12Ala and the Pro12Pro variant in the PPAR-γ gene and the response rate to pioglitazone therapy.

In our population, we show that the major confounding factors for TZD treatment efficacy are high fasting plasma glucose (>11.0 mmol/l) at baseline, HbA_{1c} value >9.0%, BMI >33 kg/m^2, and hyperinsulinemia (C-peptide concentrations at baseline >2.5 pmol/l) rather than the PPAR-γ genotype. These findings are in accordance with the previous observation that the glucose-lowering effects of TZD are dependent on the degree and duration of the decompensation of glucose metabolism (9,11,33,34). Therefore, comparisons of response rates to TZD should be based on the same initial glycemic status. Because there is evidence for a PPAR-γ gene–nutrient interaction (35), we cannot exclude an effect of individual differences in the dietary regimen on our study results. In our study population, we excluded an effect of several influence factors, such as differences in the initial glycemic control (fasting plasma glucose, HbA_{1c}), age, BMI, and duration of diabetes on the impact of a potential association between PPAR-γ genotype and the response rate to pioglitazone treatment by introducing matched subgroups for these factors. However, we could not find evidence of an association between the pioglitazone therapy response and the PPAR-γ genotype in any of these matched groups. Furthermore, the multivariate regression analysis could not identify the Pro12Ala or the Ala12Ala genotype as independent modulating factors of the pioglitazone therapy effect. Because C-peptide concentrations were significantly lower in 81.5% of the patients after pioglitazone treatment, a significant decrease in fasting hyperinsulinemia, as determined by fasting C-peptide levels, might be a better parameter for testing the therapy efficacy of TZD in patients with type 2 diabetes. Moreover, it was recently shown that pioglitazone treatment has beneficial effects on serum lipid concentrations in patients with type 2 diabetes (36,37). In accordance with these studies, we found decreased serum triglyceride concentrations and increased HDL cholesterol concentrations, independent of the genotype of the PPAR-γ gene in position 12, suggesting that these variants do not modify the beneficial effects of pioglitazone treatment on the lipid profile in our patients.

In conclusion, the Pro12Ala and the Pro12Pro variants in the PPAR-γ gene are not associated with the response rate to pioglitazone treatment in patients with type 2 diabetes. Further studies are necessary to investigate the potential importance of the Ala12Ala variant in the response to thiazolidinedione treatment. We identified initial fasting plasma glucose >11.0 mmol/l, HbA_{1c} value >9.0%, BMI >33 kg/m^2, and fasting C-peptide concentrations at baseline >2.5 pmol/l, but not the PPAR-γ genotype in position 12, as predominant confounding factors for the responder frequency to pioglitazone treatment.

Acknowledgments— We thank Dr. Michael Ristow, Klinik II und Poliklinik für Innere Medizin, Universität zu Köln, Germany, for the positive control samples of the Pro115Gln mutation. We also thank all of the patients for their participation in the study.

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