Peroxisome Proliferator–Activated Receptor-γ2 Polymorphism Pro12Ala Is Associated With Nephropathy in Type 2 Diabetes

The Berlin Diabetes Mellitus (BeDiaM) Study

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The Pro12Ala polymorphism of the gene encoding the peroxisome proliferator–activated receptor (PPAR)-γ2 has recently been shown to be associated with type 2 diabetes. In the present analysis, we investigated whether PPAR-γ2 Pro12Ala was associated with microvascular complications of type 2 diabetes, such as albuminuria, end-stage renal failure (ESRF), or retinopathy. A total of 445 patients with type 2 diabetes who were enrolled in the Berlin Diabetes Mellitus Study and in whom we determined albuminuria and the presence of ESRF and retinopathy were genotyped for the PPAR-γ2 Pro12Ala polymorphism. We also measured potentially important covariables, such as blood pressure, BMI, duration of diabetes, glycosylated hemoglobin, serum creatinine, and serum lipids. Among 445 patients with type 2 diabetes (mean age 59.3 years), the Pro12Ala genotype distribution was in Hardy-Weinberg equilibrium ($P = 0.42$). The Ala12 allele frequency was 0.14. With adjustment for covariables, the 118 Ala12 allele carriers had significantly lower urinary albumin excretion (UAE) than the 327 noncarriers (17.1 vs. 25.8 mg/d; $P = 0.01$). The percentage decrease in UAE observed in PPAR-γ Ala12 allele carriers relative to noncarriers ($P = 0.003$) rose from 0.2% ($P = 0.99$) to 54% ($P = 0.008$) and to 70% ($P = 0.01$) when the duration of diabetes increased from <10 years to 10–19 years and to ≥20 years, respectively. Similarly, the odds ratios of having albuminuria decreased from 1.22 ($P = 0.54$) to 0.61 ($P = 0.23$) and to 0.11 ($P = 0.007$), respectively. Among patients with type 2 diabetes, PPAR-γ2 Ala12 allele carriers had significantly lower UAE and tended to develop overt proteinuria less frequently. These observations suggest a protective effect of the Ala12 allele in relation to diabetic nephropathy. ***Diabetes*** **51: 2653–2657, 2002**

Type 2 diabetes constitutes a major health problem and in most populations substantially contributes to morbidity and mortality (1). Its complications include macrovascular disorders such as coronary artery disease (CAD) and microvascular lesions such as neuropathy, retinopathy, and nephropathy. Diabetic nephropathy, characterized by increased urinary albumin excretion (UAE) or microalbuminuria (2,3), can be viewed as a pathophysiologically complex phenotype. It is a harbinger of deleterious cardiovascular and renal outcomes (4,5). Furthermore, the incidence of end-stage renal failure (ESRF) as a consequence of diabetic nephropathy is rapidly increasing worldwide (6). One immediate challenge, therefore, is to identify risk factors that contribute not only to type 2 diabetes as such but also to disease-related phenotypes, such as diabetic nephropathy. Indeed, identification of common genetic factors that influence outcome might help health care providers in the early detection and treatment of potentially life-threatening disease conditions.

Diabetic nephropathy (7), as well as higher UAE rates, clusters among offspring of patients with type 2 diabetes (8); the risk of developing diabetic nephropathy has been linked to different chromosomes, including chromosome 3 (9,10), to which the peroxisome proliferator–activated receptor (PPAR)-γ2 gene has been mapped. PPAR-γ2, expressed in vascular smooth muscle cells, mesangial cells, and macrophages, is a ligand-activated transcription factor and a member of the steroid receptor superfamily (11), which has been shown to be involved in lipid and glucose metabolism, fatty acid transport, and cell differentiation (12). Most interesting is that recently developed compounds such as the thiazolidinediones (TZDs) act as PPAR-γ ligands (13,14) and have been reported to decrease albuminuria in patients with early diabetic nephropathy (15), possibly pointing to a role of PPAR-γ in the development of this disease-associated microvascular phenotype. We therefore investigated whether Pro12Ala, a recently identified functional genetic polymorphism in the PPAR-γ2 gene and previously reported to be associated with type 2 diabetes (16), was also related to type 2

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BeDiaM Study, Berlin Diabetes Mellitus Study; CAD, coronary artery disease; CI, confidence interval; ESRF, end-stage renal failure; HbA_{1c}, glycosylated hemoglobin; PAI, plasminogen-activator inhibitor; PPAR, peroxisome proliferator–activated receptor; TZD, thiazolidinedione; UAE, urinary albumin excretion.

TABLE 1
Characteristics of 445 patients with diabetes

	Men (n = 220)	Women (n = 225)
Age (years)	57.8 ± 9.8	60.6 ± 10.3*
BMI (kg/m^2)	27.6 ± 4.1	28.5 ± 5.5
Duration of diabetes (years)	10 (4–16)	10 (5–17)
Insulin treatment	139 (63.2)	161 (71.6)
Intake of antidiabetic drugs	131 (61.4)	121 (53.8)
HbA_{1c} (%)	8.3 ± 1.7	8.4 ± 1.8
Serum creatinine (μmol/l)	73.6 (71.3–75.9)	61.3 (59.0–63.6)*
UAE (μg/min)	24.8 (20.3–30.2)	21.8 (18.0–26.5)
Macroalbuminuria	94 (42.7)	103 (45.8)
ESRF	30 (13.6)	14 (6.2)*
Retinopathy	36 (16.7)	33 (15.1)
Hypertension	80 (36.4)	120 (53.3)*
Systolic blood pressure (mmHg)	137.3 ± 17.2	140.9 ± 21.3
Diastolic blood pressure (mmHg)	80.0 ± 8.1	80.6 ± 10.7
Serum total cholesterol (mmol/l)	5.8 ± 1.2	5.9 ± 1.2
Serum triglycerides (mmol/l)	5.3 ± 3.3	4.7 ± 3.4
Cardiovascular complications	52 (23.6)	53 (23.6)

Data are arithmetic means ± SD, medians (interquartile range) (for duration of diabetes), geometric means (95% CI for serum creatinine and UAE), or n (%). Serum lipids were measured in 172 men and 190 women. *$P < 0.01$ vs. men.

diabetes–associated disease phenotypes in patients of European ancestry.

RESEARCH DESIGN AND METHODS

Patient selection and clinical investigation. Patients who had type 2 diabetes (n = 445) and were of Caucasian ethnicity were recruited from three diabetes and two dialysis centers as well as two general hospitals in Berlin within the framework of the Berlin Diabetes Mellitus (BeDiaM) Study. Classification of type 2 diabetes relied on the revised diagnostic criteria recommended by the American Diabetes Association (17). The diagnosis of nephropathy was based on repeated observation of UAE of >20 μg/min in nonoliguric patients or on the presence of ESRF necessitating renal replacement therapy (dialysis or transplantation). Macroalbuminuria was defined as UAE >200 μg/min. Other causes of increased albumin excretion were excluded by appropriate clinical and technical investigation. In agreement with recently published World Health Organization criteria (18), hypertension was defined as a systolic pressure of ≥140 mmHg or diastolic pressure of ≥90 mmHg on at least two separate occasions or as the necessity to prescribe antihypertensive drugs, irrespective of the blood pressure level. An investigator unaware of the patient's genotype searched the medical records for history of CAD, stroke, or retinopathy. The diagnosis of CAD required a history of myocardial infarction, coronary angioplasty or bypass surgery, coronary lesions on angiography, or treatment with nitrates or a clinical history of angina pectoris. Retinopathy was defined as stages III or IV on funduscopy or as history of laser therapy. Serum creatinine, serum lipids, and glycosylated hemoglobin (HbA_{1c}) were determined by standard laboratory techniques.

The study protocol was approved by the institutional Ethics Committee. All subjects gave informed consent before their enrollment in the study.

Statistical analysis. We used SAS version 8.1 (SAS Institute, Cary, NC) for database management and statistical analysis. Measurements with a skewed distribution were normalized by logarithmic transformation. Comparisons of means and proportions were performed with the standard normal Z test and Fisher's exact test, respectively. Significant covariables were traced by stepwise linear or logistic regression. We used analysis of covariance to compare adjusted continuous measurements between genotypes. The presence of mild renal dysfunction in relation to genotype was studied by multiple logistic regression.

Genotyping. Genomic DNA was prepared from peripheral blood using a DNA-selective preparation method (Qiagen, Hilden, Germany). The region encompassing the Pro12Ala polymorphic site was amplified by PCR as previously described (16). PCR products were digested using the restriction enzyme *BstU*-1 and visualized on ultraviolet-transilluminated ethidium bromide–stained agarose gels.

RESULTS

PPAR-γ2 Pro12Ala polymorphism is related to UAE and macroalbuminuria in patients with type 2 diabetes. The clinical characteristics of 220 men and 225 women with type 2 diabetes are presented in Table 1. On average, women were older than men and had a higher serum creatinine concentration. Women were more frequently hypertensive but in fewer instances proceeded to ESRF.

The genotypic distribution of the PPAR-γ2 Pro12Ala gene polymorphism was in Hardy-Weinberg equilibrium (P = 0.42), and the Ala12 allele frequency in our diabetes population was 0.14 (Table 2).

Among our patients with type 2 diabetes, the 118 Ala12 carriers showed a significantly lower UAE than the 327 noncarriers (17.1 μg/min [95% confidence interval (CI) 13.5–21.8 μg/min] vs. 25.8 μg/min [95% CI 21.9–30.4 μg/min]; P = 0.01) (data not shown). With adjustment for sex, age, BMI, hypertension, hyperlipidemia, duration of diabetes, and treatment with insulin or oral antidiabetic drugs, the percentage difference was 32% (95% CI 8–50%; P =

TABLE 2
Genotype and allele frequencies in patients with and without microvascular complications

	ProPro	ProAla	AlaAla	P*	Pro	Ala	P*
No microvascular complications	144 (70.9)	55 (27.1)	4 (2.0)	—	343 (84.5)	63 (15.5)	—
Macroalbuminuria	154 (78.2)	37 (18.8)	6 (3.0)	0.12	345 (87.6)	49 (12.4)	0.22
ESRF	30 (68.2)	13 (29.5)	1 (2.3)	0.88	73 (83.0)	15 (17.0)	0.75
Retinopathy	55 (79.7)	13 (18.8)	1 (1.5)	0.33	123 (89.1)	15 (10.9)	0.20

Data are n (%). *For comparison with group without microvascular complications.

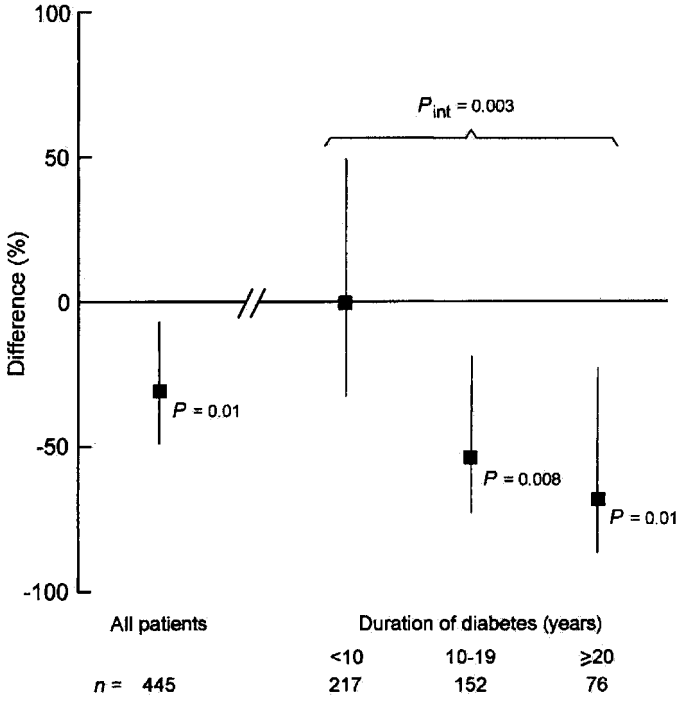

FIG. 1. Percentage difference in UAE between PPAR-γ2 Ala12 allele carriers and noncarriers for all patients and for subgroups according to duration of diabetes. Differences were adjusted for sex, age, BMI, hypertension, hyperlipidemia, duration of diabetes, and treatment with insulin or oral antidiabetic drugs. Vertical lines denote 95% CI. For each subgroup, the number of subjects is given (bottom). Number of patients (*n*) and significance levels for the difference (*P*) and for the interaction (P_{int}) between the genetic polymorphism and duration of diabetes are given.

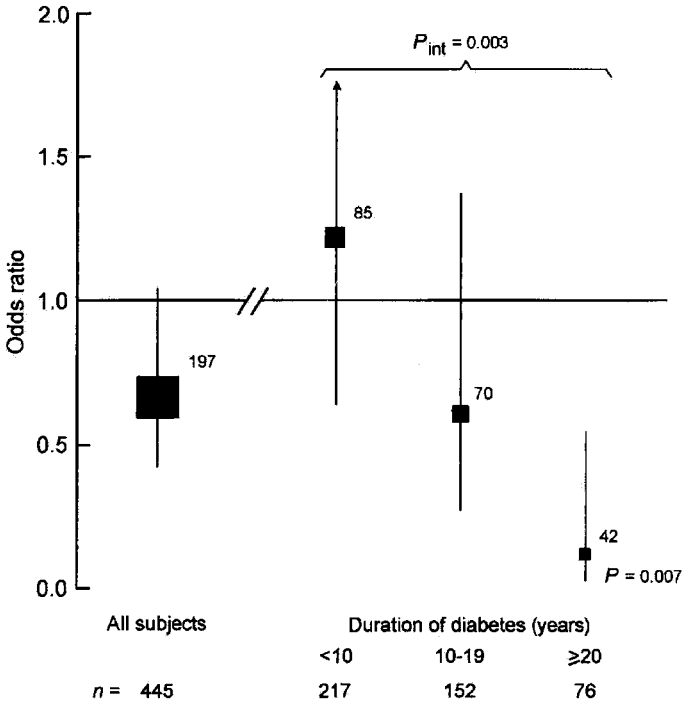

FIG. 2. Odds ratios of having macroalbuminuria in PPAR-γ2 Ala12 allele carriers versus noncarriers for all patients and subgroups according to duration of diabetes. The odds ratios were adjusted for sex, age, BMI, hypertension, hyperlipidemia, duration of diabetes, and treatment with insulin or oral antidiabetic drugs. Vertical lines denote 95% CI. The size of the symbols is proportional to the number of cases given alongside the squares. Number of patients (*n*) and significance levels for odds ratios (*P*) and for the interaction (P_{int}) between the genetic polymorphism and duration of diabetes are given.

0.01) (Fig. 1). Furthermore, the percentage difference in UAE between Ala12 allele carriers and noncarriers increased ($P = 0.003$) with longer duration of diabetes (Fig. 1). With similar adjustments as mentioned above, the percentage difference was 54% in patients with diabetes and a disease history from 10 to 19 years (95% CI 20–74%; $P = 0.008$) and 70% in those who had diabetes for ≥20 years (95% CI 24–88%; $P = 0.01$). In contrast, among those patients in whom diabetes was diagnosed within the past 10 years, there was no difference in UAE with respect to the different Pro12Ala genotypes ($P > 0.99$) (Fig. 1).

We also calculated the odds ratio of having proteinuria coded as a dichotomous trait (0, 1) with adjustments for the same covariables as in the continuous analysis. In all 445 patients, the odds ratio was 0.66 for Ala12 allele carriers compared with noncarriers (95% CI 0.42–1.04; $P = 0.07$) (Table 3), which just failed to reach statistical significance because of a significant interaction ($P = 0.003$) (Fig. 2) between the Pro12Ala polymorphism and the duration of diabetes. Indeed, the odds ratio decreased from 1.22 (95% CI 0.64–2.32; $P = 0.54$) to 0.11 (95% CI 0.02–0.55; $P = 0.007$) in patients in whom the disease history was <10 years compared with those who had diabetes for ≥20 years.

There was no significant association of the PPAR-γ2 Pro12Ala polymorphism with any other measured phenotype in the BeDiaM Study, including ESRF or diabetic retinopathy, before and after allowing for the duration of diabetes.

DISCUSSION

The main finding of the present study was that among patients with type 2 diabetes, PPAR-γ2 Ala12 allele carriers had significantly lower UAE and presented less frequently with macroalbuminuria. This suggests a role of genetic variation in the PPAR-γ2 gene in the development of diabetes-related nephropathy. The strength of the association increased with longer duration of diabetes, a phenotype that is likely to represent a surrogate of environmental factors that over time modulate the genetic

TABLE 3
Association between microvascular complications of diabetes and the PPAR-γ2 Pro12Ala polymorphism

	ProPro (*n* = 327)	AlaAla+AlaPro (*n* = 118)	Relative risk (95% CI)*	*P*
Macroalbuminuria	47.1 (154)	36.4 (43)	0.66 (0.42, 1.04)	0.07
ESRF	9.2 (30)	11.9 (14)	1.27 (0.63, 2.57)	0.51
Retinopathy	16.8 (55)	11.9 (14)	0.64 (0.32, 1.27)	0.20

Data are % (*n*) unless otherwise indicated. *The odds ratios of having microvascular disease (ProAla+AlaAla versus ProPro) were adjusted for sex, age, BMI, hypertension, hyperlipidemia, duration of diabetes, and treatment with insulin or oral antidiabetic drugs.

impact on diabetic nephropathy. The reason that we were not able to show an association between ESRF and Pro12Ala in our patients with type 2 diabetes might be related to the fact that ESRF is a more complex phenotype that is determined by a plethora of different pathophysiological as well as genetic factors. However, patients with type 2 diabetes have an excessive cardiovascular risk, leading to a dramatically reduced survival before ESRF develops (6,19), therefore introducing a bias toward low ESRF rates that hampers the unraveling of the underlying genetic risk factors. In the development of diabetic nephropathy, morphologic changes such as thickening of the glomerular basement membrane, glomerular hypertrophy, mesangial expansion, and modest expansion of the tubulointerstitium play a role. All of these factors lead to microalbuminuria as a diagnostic hallmark of diabetic nephropathy, preceding the development of overt proteinuria (20).

The risk of developing diabetic nephropathy is likely to be genetically determined, because not all patients share the same renal outcome even when obviously presenting with the same degree of metabolic dysregulation. In this regard, the prevalence of microalbuminuria has been reported to be significantly higher in Mexican-American than in non-Hispanic white diabetic subjects (21,22), and higher UAEs have been observed in offspring of patients with type 2 diabetes (23). Strojek et al. (8) recently reported higher UAE rates in healthy offspring of diabetic parents with nephropathy versus those without nephropathy. Furthermore, the risk of developing diabetic nephropathy has been linked to different chromosomes, including chromosome 3 (10), on which the gene encoding PPAR-γ2 is located.

Recently developed compounds such as TZDs act as potent PPAR-γ ligands and have been shown to ameliorate microalbuminuria in patients with early diabetic nephropathy (15). Nakamura et al. (24) recently observed that TZDs reduce UAE in microalbuminuric patients as well as the serum concentration of type IV collagen, whereas glibenclamide showed no such effects. Other investigators reported that TZDs lower microalbuminuria, possibly by inhibiting platelet-derived growth factor–stimulated cell growth and angiotensin II–induced plasminogen-activator inhibitor (PAI)-1 expression, both of which are known to contribute to the development of nephropathy (25).

The mechanisms by which PPAR-γ2 and variation in its gene might actually protect against diabetic nephropathy remain to be elucidated. Even if glomerular expression of PPAR-γ has been reported (26,27), the major sites of PPAR-γ2 expression are adipocytes (28). Adipocytes generate and secrete PAI-1 (29), angiotensin II, and type IV collagen (30). In this respect, it is conceivable that a reduced level of PPAR-γ2 activation in preadipocytes, leading to reduced adipocyte differentiation, may be responsible for reduced levels of PAI-1, angiotensin II, and type IV collagen in patients who carry the Ala12 allele of PPAR-γ2, possibly resulting in renal protection.

In agreement with several other association studies (31–33), on the PPAR-γ2 Pro12 allele and type 2 diabetes, our results speak in favor of a protective effect of the Ala12 allele on diabetes-associated phenotypes, i.e., nephropathy. The molecular mechanism by which the Ala12 carrying PPAR-γ2 protein might achieve these beneficial actions, which largely resemble the specific effects of TZDs on diabetic nephropathy, remains to be elucidated.

In conclusion, among patients with type 2 diabetes, PPAR-γ2 Ala12 allele carriers have significantly lower UAE and develop overt proteinuria less frequently. These observations suggest a protective effect of the Ala12 allele in relation to diabetic nephropathy. Whether TZDs as PPAR-γ ligands might be more effective in patients with type 2 diabetes who carry the PPAR-γ2 Pro12 risk allele remains to be investigated in future clinical studies.

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