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Association of Prostaglandin E Synthase 2 (*PTGES2*) Arg298His Polymorphism with Type 2 Diabetes in Two German Study Populations

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Context: On the basis of its chromosomal localization and its role in the synthesis of the antilipolytic compound prostaglandin E_2, the prostaglandin E synthase 2 (*PTGES2*) is a candidate gene for type 2 diabetes.

Objective: The aim of the present study was to investigate whether genetic variants in the *PTGES2* gene are associated with type 2 diabetes.

Results: Sequencing of the *PTGES2* gene revealed one nonsynonymous coding single-nucleotide polymorphism (SNP) (Arg298His, rs13283456) and a previously unknown promoter SNP g.-417G>T. Both SNPs and additional haplotype tagging SNPs (rs884115, rs10987883, rs4837240) were genotyped in a nested case-control study of 192 incident type 2 diabetes subjects and 384 controls (European Prospective Investigation into Cancer and Nutrition-Potsdam). Carriers of the minor allele of Arg298His had a lower risk to develop the disease [odds ratio (OR) 0.63, 95% confidence interval (CI) 0.41–0.97, $P = 0.04$], compared with homozygous individuals with the common allele. The *PTGES2* Arg298His polymorphism was reinvestigated in a population-based cross-sectional study (Cooperative Health Research in the Augsburg Region) consisting of 239 individuals with impaired glucose tolerance, 226 with type 2 diabetes, and 863 normoglycemic controls. In this study population, the Arg298His polymorphism was significantly associated with impaired glucose tolerance (OR 0.68, 95% CI 0.50–0.93, $P = 0.007$) and type 2 diabetes (OR 0.61, 95% CI 0.43–0.86, $P = 0.004$). A pooled analysis of data from both study populations revealed reduced risk of type 2 diabetes (OR 0.62, 95% CI 0.47–0.81, $P = 0.0005$) in *PTGES2* 298His allele carriers.

Conclusion: We obtained evidence from two Caucasian study populations that the His298-allele of *PTGES2* Arg298His confers to reduced risk of type 2 diabetes. **(*J Clin Endocrinol Metab* 92: 3183–3188, 2007)**

PROSTANGLANDIN (PG) E_2 is widely distributed in various organs, and exhibits several biologically important activities such as smooth muscle dilatation/contraction, sodium excretion, body temperature regulation, induction of pain, stimulation of bone resorption, and inhibition of immune responses (1, 2). Besides these functions, PGE_2 is a potent antilipolytic compound in human adipose tissue (3–7). Different cell types of the adipose tissues including endothelial cells and adipocytes form and release PGE_2, which exhibits its paracrine and autocrine antilipolytic activity via the high affinity to PGE_2 receptor EP3 (4, 8, 9). Similar mechanisms were discussed for the regulation of leptin release by PGE_2 (10, 11). PGE_2 may also contribute to the excessive development of adipose tissue mass by means of hypertrophy (4, 12).

The synthesis of PGE_2 from arachidonic acid is mediated by phospholipase A_2, cyclooxygenase [prostaglandin E synthase 2 (PTGES2)] and prostaglandin E synthase (PTGES). Terminal PTGESs, which catalyze the conversion of PGH_2 to PGE_2, exists in three forms (2): microsomal PTGES (PTGES1), cytosolic PTGES, and membrane-bound PTGES2. Whereas the physiological role of cytosolic PTGES is uncertain (2), PTGES1 seems to be mainly involved in inflammation (13–15). In contrast, PTGES2 is not induced by inflammatory stimuli and is expressed constitutively in various cells and tissues in which PTGES1 expression is relatively low (16, 17).

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Abbreviations: BMI, Body mass index; CI, confidence interval; EPIC, European Prospective Investigation into Cancer and Nutrition; HOMA, homeostasis model assessment; HOMA-%B, HOMA % β-cell function; HOMA-IR, HOMA insulin resistance index; htSNP, haplotype tagging SNP; HWE, Hardy-Weinberg equilibrium; IGT, impaired glucose tolerance; KORA, Cooperative Health Research in the Augsburg Region; KORA S4, KORA Survey 4; LD, linkage disequilibrium; OGTT, oral glucose tolerance test; OR, odds ratio; PG, prostaglandin; PTGES, prostaglandin E synthase; PTGES1, microsomal PTGES; PTGES2, prostaglandin E synthase 2; SNP, single-nucleotide polymorphism; UTR, untranslated region.

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These findings argue for a general role of PTGES2 in the production of PGE_2 crucial for tissue homeostasis.

The *PTGES2* gene maps close to chromosome region 9q34.13, which showed nominal significant ($P = 0.001$) linkage to body weight (18). Considering the chromosomal localization of *PTGES2* and its role in the synthesis of the antilipolytic PGE_2, we proposed that *PTGES2* is a functional candidate gene for type 2 diabetes or other traits of the metabolic syndrome. Therefore, we screened exons 1–7 (377 amino acid residues of *PTGES2* isoform 1, NM_025072), including exon-intron boundaries and the promoter region of *PTGES2* for sequence variations (19). The identified coding single-nucleotide polymorphism (SNP), a putative regulatory SNP, and in addition, all haplotype tagging SNPs of the 20-kb gene region were analyzed for association with type 2 diabetes and related phenotypes of the disease in two Caucasian study populations [(European Prospective Investigation into Cancer and Nutrition (EPIC) and Cooperative Health Research in the Augsburg Region (KORA)].

Subjects and Methods

EPIC Potsdam study

Nested case-control study subjects were taken from the EPIC-Potsdam cohort. This population-based, prospective study comprises a total of 27,548 people from the area around Potsdam, Germany. Baseline examinations were conducted between 1994 and 1998 and included anthropometric and blood pressure measurements, blood sampling, a self-administered food-frequency questionnaire, and a personal interview on lifestyle habits and medical history (20, 21). During the first follow-up period, on average 2–3 yr after recruitment, 192 newly diagnosed cases of type 2 diabetes [International Classification of Diseases 10:E11: Not primary insulin-dependent diabetes mellitus (type 2 diabetes)] were identified by self-report and confirmed by the patients primary care physician. Type 1 diabetes-associated antibodies glutamic acid decarboxylase-65 and insulinoma-associated antigen-2 were analyzed in all blood samples belonging to the verified case subjects. Cases were then matched with two control subjects from the basic cohort each by age (± 1 yr) and sex (n = 384). Characteristics of the study population have been described in detail before (22, 23) In brief, gender distribution of the case-control study was 59% male and 41% female subjects with a mean age of 55.5 yr (35–65 yr). Incident cases had significantly higher body mass index (BMI), waist to hip ratio, C-reactive protein and hemoglobin A1c levels, lower high-density lipoprotein cholesterol and adiponectin levels, and higher prevalence of hyperlipidemia and hypertension and showed less sports activity at baseline. Baseline blood pressure readings were available from 376 (65%) study subjects who did not report any antihypertensive medications taken during the previous 4 wk. All study participants had given informed consent, and the genotype assessment was agreed to by the local ethics committee.

KORA

The KORA Survey 4 (KORA S4) studied a population-based sample of 4261 subjects aged 25–74 yr during 1999–2001 (24). Each study participant signed a consent form to participate in genetic studies. All study methods were approved by the ethics committee of the Bavarian medical association, Munich. The sampling design followed the guidelines of three previous surveys in the same region as part of the multinational World Health Organization-Monitoring Trends and Determinants of Cardiovascular Disease study. In the age range of 55–74 yr, 1653 people participated in a standardized interview followed by biochemical and clinical analyses. An oral glucose tolerance test and biochemical and immunological analyses were performed as described previously (25). Acute infections (fever) or gastrointestinal illness were an exclusion criterion for the oral glucose tolerance test. Diabetes was diagnosed according to 1999 World Health Organization criteria (25). After exclusion of all subjects with self-reported type 1 diabetes, humoral autoimmunity to glutamic acid decarboxylase, or diabetes onset in the context of pancreatitis, a total of 226 individuals with type 2 diabetes and 239 individuals with impaired glucose tolerance (IGT) were available for analyses. There were 863 normoglycemic control subjects randomly selected in the same age range. This totaled 1328 probands. Of the diabetic patients, 120 were newly detected and did not yet receive antidiabetic treatment; of the other 116, 33% were under insulin treatment and 57% took oral antidiabetic agents (25).

Sequencing, selection of haplotype tagging SNPs (htSNPs), and genotyping

Sequence information of the *PTGES2* gene (ID 80142) was derived from GenBank (www.ncbi.nlm.nih.gov). For sequencing, DNA was isolated from 47 unrelated subjects from the Metabolic Intervention Cohort Kiel. For extraction E.Z.N.A. Blood DNA minikit (Peqlab Biotechnologie GmbH, Erlangen, Germany) was used according to the manufacturer's instructions. Seven exons, including exon-intron boundaries and 822 bp from the 5′ untranslated region (UTR)/promoter region of *PTGES2* were analyzed by terminator cycle sequencing using Big Dye chemistry on an ABI 3700 capillary DNA sequencer (Applied Biosystems, Foster City, CA). Amplifications were performed using a Touch-Down PCR by decreasing the annealing temperature three times by 2 C, starting 2 C above the specific primer temperature. The sequence data were analyzed by Lasergene sequence analysis software (DNASTAR, Inc., Madison, WI). For htSNP selection, Haploview 3.2 was used to analyze a 20-kb region comprising *PTGES2* (chromosome 9: position 127.952.200–127.972.200). htSNPs with a minor allele frequency greater than 0.1 (rs10987883, rs4837240, rs884115) were obtained from CEPH HapMap data release 21 (www.hapmap.org).

Genotyping of the 576 EPIC subjects was performed with the TaqMan system (ABI Prism 7900 HT). The success rate of genotyping was greater than 99.7%. Genotyping of the KORA S4 study group from Augsburg was performed in the Genome Analysis Center of the National Research Center for Environment and Health (GSK) using the Mass-ARRAY system (Sequenom, San Diego, CA) as described previously (26). The success rate of genotyping was greater than 99.9%. Sequences of primers and assay probes are available on request.

Western blot analysis

Protein extracts from human skeletal muscle (BioCat, Heidelberg, Germany), liver, and Huh7, HepG2, CaCo2, LNCaP, HeLa, and SGBS cells [adipocyte cell line, (27)] as well as cytosolic and mitochondrial fractions were separated by SDS-PAGE (12–20%) using the XCell-SureLock Mini Gelsystem (Invitrogen, Karlsruhe, Germany), transferred to polyvinyl difluoride membrane, and probed (dilution 1:7500) with a polyclonal anti-PTGES2 antibody (Cayman Chemical, Ann Arbor, MI).

Population stratification

The EPIC-Potsdam study was tested for stratification or admixture using the 384 control subjects according to Pritchard *et al.* (28). For each individual, 42 SNPs were typed. The SNPs were spread over 18 chromosomes and localized in different genes respectively, implying that there is no linkage disequilibrium between these markers. All SNPs had minor allele frequencies greater than 25%. The estimated probabilities that the observed genotype frequencies originated from more than one population were very small ($<10^{-20}$). Therefore, we concluded that there are no major admixture effects within the EPIC-Potsdam study. In the KORA study, we performed two genomic control studies to test of population stratification. In the first one, we genotyped more than 700 subjects from KORA (southwest Germany) and compared them with subjects of two different population-based studies from the northeast and northwest of Germany. Altogether we genotyped 210 SNP markers in genomic regions not known to be under genetic selection (29). Second, we submitted a manuscript including a genomic control study with 530 KORA S4 subjects (Winkelmann, J., P. Lichtnerl, B. Schormairl, M. Uhr, S. Hauk, K. Stiasny-Kolster, C. Trenkwalder, W. Paulus, I. Peglau, I. Eisensehr, T. Illig, H. E. Wichmann, H. Pfister, J. Golic, T. Bettecken, B. Pütz, F. Holsboer, T. Meitinger, and B. Müller-Myhsok, submitted for publication). Neither of the two studies showed major population stratification. Although only subpopulations of our study subjects were

TABLE 1. Minor allele frequencies and allelic association with type 2 diabetes of *PTGES2* SNPs in EPIC-Potsdam

SNP	Minor allele	Cases	Controls	*P* value HWE	*P* value[a]
g.-417G>T	T	0.029	0.027	0.14	0.85
rs884115	T	0.116	0.114	0.01	
Arg298His	A	0.152	0.194	0.79	0.08
rs10987883	G	0.118	0.124	0.43	0.77
rs4837240	A	0.178	0.164	0.12	0.57

[a] Cochran Armitage trend test.

analyzed in these genomic control studies, we are quite confident that there is no major population stratification in the KORA cohort.

Statistical analyses

Statistics were computed with SAS software 9.1 (SAS Institute, Cary, NC) and S-PLUS 6.2 professional edition (Insightful Corp., Seattle, WA). Allele and genotype frequencies were determined by gene counting. Control subjects of both study populations were tested for the distribution of genotypes according to the Hardy-Weinberg equilibrium (HWE) with a χ^2 test. Comparison of genotypes in cases and controls was calculated by Armitage's trend statistics (1 degree of freedom). *P* values in the range of 0.05 to greater than 0.1 were considered for further analyses. Haplotype frequencies in type 2 diabetic subjects and controls were estimated by maximum likelihood methods using SASGenetics software (SAS Institute). Linkage disequilibria (Lewontin's *D′* and linkage coefficient r^2) were generated by likelihood ratio tests. Individual probabilities of haplotypes (frequency > 1%) were estimated using haplotype trend regression method (30). Output data from the haplotype trend regression were fitted in logistic regression procedures. Crude and adjusted odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were determined by logistic regression analysis in codominant and dominant inheritance models. Pooled analysis of genotype data from both study populations was performed according to Hardy and Thompson (31). Genotype differences in anthropometric, blood pressure measurements, and homeostasis model assessment (HOMA) values after log transformation [HOMA % β-cell function (HOMA-%B), HOMA insulin resistance index (HOMA-IR)] were analyzed by linear regression analysis. All values were computed in an unadjusted model for all nondiabetes cases. Association to blood pressure is computed in all controls and diabetes cases without treatment of hypertension. Models on HOMA-%B and HOMA-IR are computed with log-transformed outcomes.

Results

SNP identification and selection

A total of 2710 bp including all seven exons, exon-intron boundaries, and 5′ UTR/promoter region (822 bp) of the *PTGES2* gene (NM_025072) were sequenced in 94 chromosomes of unrelated subjects (19). No splice-site alteration was found. One coding SNP (rs13283456, C>T) and a previously unknown promoter SNP (g.-417G>T) were identified. Other coding SNPs from National Center for Biotechnology Information SNP database (rs204004, rs1537573) were not found. The coding SNP rs13283456 results in an arginine (Arg) to histidine (His) change at position 298 (NP_079348) of PTGES2. To investigate a possible association between variants of the *PTGES2* gene and type 2 diabetes or phenotypes related to this disease, the Arg298His variant and the promoter SNP (g-417G>T) as well as the haplotype tagging SNPs from the CEPH HapMap database (rs4837240, rs10987883, rs884115) of the 20-kb gene region were genotyped in the nested case-control study of 192 incident type 2 diabetes patients and 384 controls from the EPIC-Potsdam cohort. SNP rs884115 showed deviation from HWE ($P = 0.01$) (Table 1) and therefore was excluded from further analyses. Armitage's trend test revealed borderline association between the *PTGES2* Arg298His polymorphism and type 2 diabetes (Table 1). The other polymorphisms showed no association with the disease.

Association analysis in EPIC-Potsdam

The pairwise linkage disequilibrium (LD) pattern of *PTGES2* SNPs is shown in Table 2. htSNPs rs483720 and rs10987883 were in high LD with promoter SNP g.-417G>T ($D' = 1.0$). Arg298His showed a low degree of LD with promoter SNP g.-417G>T ($D' = 0.45$) and moderate LD ($' \cong 0.8$) with the other two SNPs, respectively. The haplotype frequencies in type 2 diabetic subjects and controls are shown in Table 3. A common haplotype (II) containing the minor allele of *PTGES2* Arg298His and the major alleles of *PTGES2* htSNPs, and g.-417G>T showed reduced risk of type 2 diabetes, but this difference did not reach significance (OR 0.51, 95% CI 0.23–1.11, $P = 0.09$). Single SNP analysis of association with disease status (Table 4) revealed a risk reducing effect of the His298 allele similar to that of haplotype II. In comparison with subjects homozygous for the major allele (Arg/Arg), carriers of the rare allele (Arg/His+His/His) had significantly lower risk of type 2 diabetes (OR 0.63, 95% CI 0.41–0.97, $P = 0.04$) in the adjusted model. Polymorphisms rs4837240, g,-417G>T, and rs10987883 were not associated with the disease (data not shown).

Verification study in KORA

The association between the *PTGES2* Arg298His polymorphism and type 2 diabetes was also studied in a second population-based study taken from KORA S4. Based on an oral glucose tolerance test (OGTT), 239 individuals with IGT

TABLE 2. Linkage coefficient (r^2) and Lewontin's *D′* of the *PTGES2* SNPs in EPIC-Potsdam

		D′			
		g.-417G>T	Arg298His	rs10987883	rs4837240
r^2	g.-417G>T		0.450	1.000	1.000
	Arg298His	0.001		0.757	0.810
	rs10987883	0.004	0.018		1.000
	rs4837240	0.006	0.030	0.705	

TABLE 3. PTGES2 haplotype frequencies and associations with type 2 diabetes in EPIC-Potsdam

No.	Haplotype	Cases	Controls	OR (95% CI)[a]	*P*
I	1–1-1–1	0.644	0.626	1.0 (reference)	
II	1–2-1–1	0.151	0.183	0.51 (0.23–1.11)	0.09
III	1–1-2–2	0.120	0.118	0.94 (0.39–2.29)	0.89
IV	1–1-1–2	0.056	0.037	2.28 (0.63–8.21)	0.21
V	2–1-1–1	0.029	0.023	0.71 (0.13–3.84)	0.69

For the haplotype designation, the allele with the higher prevalence is denoted with 1, the minor allele with 2. The order of polymorphisms corresponds to their position in the 5′ to 3′ direction of the gene: g.-417G>T, Arg298His, rs10987883, and rs4837240.

[a] Adjusted for sex, age, and BMI.

and 226 individuals with type 2 diabetes were available for association analyses. A total of 863 normoglycemic subjects served as controls. In Table 4, genotype frequencies and relative risk estimates (ORs) of the *PTGES2* Arg298His polymorphism in KORA are shown. Logistic regression analyses revealed significant associations with IGT and type 2 diabetes. For individuals with the minor allele, we observed a decreased risk for developing IGT and type 2 diabetes with adjusted ORs of 0.68 (95% CI 0.50–0.93, $P = 0.007$) and 0.61 (95% CI 0.43–0.86, $P = 0.004$). Significant associations were also obtained in an unadjusted model. When the IGT and type 2 diabetes group were merged into one analysis group, we obtained a crude OR of 0.66 ($P = 0.0006$) and an adjusted OR of 0.66 ($P = 0.0017$). A pooled analysis of data from both study populations revealed an OR of 0.62 (95% CI 0.47–0.81, $P = 0.0005$) for association between *PTGES2* Arg298His SNP and type 2 diabetes. We also tested for associations of this polymorphism-related phenotypes of the disease. As shown in Table 5, in control subjects but not cases, we found significant lower HOMA-%B ($P = 0.036$) as a measure of basal insulin secretion in minor allele carriers of *PTGES2* Arg298His. Lower HOMA-IR values were also found in control subjects, but the difference reached no significance ($P = 0.086$). Other traits showed no associations to the SNP.

Expression of the PTGES2 protein

To find out whether the PTGES2 protein is expressed in human tissues and cell types, we performed Western blot analyses. As shown in Fig. 1, the PTGES2 protein was expressed in skeletal muscles (lane 12) and liver (lane 13) as well as cell lines derived from the prostate, intestine (lanes 8 and 9), and liver (lanes 1, 6, and 7). Interestingly, PTGES2 was present in differentiated adipocytes but not preadipocytes (lanes 3–5). In HeLa cells, PTGES2 was not detectable (lane 11). The expression of PTGES2 in muscles was also shown by Tanikawa *et al.* (17).

Discussion

Because the prostaglandin E synthase 2 (*PTGES2*) gene maps to a chromosomal locus linked to obesity (18) and is important for synthesis of the antilipolytic-hypertrophic (3) metabolite PGE_2, we tested the hypothesis that *PTGES2* polymorphisms are associated with type 2 diabetes and related traits in two German study populations. Sequencing (19) of all seven exons of *PTGES2* revealed only one nonsynonymous SNP in codon 298 (Arg→His), which had not been genotyped in a large population before. The minor allele (His298) frequencies observed were 12.0% in our screening group comprising 47 individuals, 16.5% in EPIC, and 18.6% in KORA. The limitation of our EPIC cohort should be noted because an OGTT was not performed in this cohort. Type 2 diabetes was identified only by self-report and confirmed by the patient primary care physician. Thus, some individuals of the control group might already be in the stage of IGT or diabetes mellitus not yet diagnosed by the physician. We would expect that this bias deteriorated rather than improved the significance of the results. However, in our verification cohort (KORA), OGTTs were available from the all patients and controls. The key finding in the present study is the consistent association between the minor allele of the *PTGES2* Arg298His SNP (rs13283456) and decreased risk of diabetes type 2 in both study populations. This finding was confirmed in a subgroup of KORA consisting of 239 subjects with IGT. In addition, a pooled analysis of data from both

TABLE 4. Genotype distribution and association of *PTGES2* Arg298His genotypes with type 2 diabetes or IGT in the EPIC-Potsdam and KORA cohort

Cohort	Genotype	Cases (%)	Controls (%)	Crude OR (95% CI)	*P*	Adjusted OR[a] (95% CI)	*P*
EPIC	Arg/Arg	135 (72)	245 (65)				
T2D	Arg/His	49 (26)	116 (31)	0.77 (0.52–1.14)	0.42	0.66 (0.42–1.03)	0.07
	His/His	4 (2)	15 (4)	0.48 (0.16–1.49)	0.19	0.44 (0.13–1.48)	0.19
	Arg/His+His/His	53 (28)	131 (35)	0.73 (0.50–1.08)	0.11	0.63 (0.41–0.97)	0.04
KORA	Arg/Arg	160 (71)	519 (60)	1.0		1.0	
T2D	Arg/His	59 (26)	302 (35)	0.63 (0.46–0.88)	0.007	0.61 (0.43–0.87)	0.006
	His/His	7 (3)	42 (5)	0.54 (0.24–1.23)	0.141	0.61 (0.26–1.43)	0.258
	Arg/His+His/His	66 (29)	344 (40)	0.62 (0.45–0.85)	0.003	0.61 (0.43–0.86)	0.004
KORA	Arg/Arg	164 (69)	519 (60)	1.0		1.0	
IGT	Arg/His	71 (30)	302 (35)	0.74 (0.54–1.02)	0.064	0.73 (0.52–1.00)	0.052
	His/His	4 (1)	42 (5)	0.30 (0.11–0.85)	0.024	0.33 (0.11–0.95)	0.040
	Arg/His+His/His	75 (31)	344 (40)	0.69 (0.51–0.94)	0.006	0.68 (0.50–0.93)	0.007

Logistic regression analyses. T2D, Type 2 diabetes.

[a] Adjusted for sex, age, and BMI.

TABLE 5. Anthropometric variables, blood pressure measurements, and HOMA values according to *PTGES2* Arg298His genotypes in type 2 diabetes cases and controls in KORA

	Controls			Cases		
	Arg/Arg	Arg/His+His/His	*P*	Arg/Arg	Arg/His+His/His	*P*
Subjects (no.)	519	344		160	66	
BMI (kg/m^2)	27.66 (0.18)	27.63 (0.21)	0.928	31.16 (0.36)	30.17 (0.56)	0.139
Waist to hip ratio	0.888 (0.003)	0.881 (0.004)	0.250	0.940 (0.006)	0.939 (0.010)	0.927
Waist circumference (cm)	93.28 (0.49)	93.08 (0.60)	0.792	103.71 (0.89)	101.43 (1.38)	0.165
Systolic blood pressure (mm Hg)	130.31 (0.96)	129.16 (1.16)	0.447	145.99 (2.42)	144.80 (3.69)	0.789
Diastolic blood pressure (mm Hg)	78.75 (0.51)	77.55 (0.61)	0.133	83.71 (1.27)	83.58 (1.94)	0.954
Log (HOMA-%B)	0.73 (0.03)	0.64 (0.03)	0.036	0.94 (0.06)	1.09 (0.10)	0.207
Log (HOMA-IR)	0.80 (0.03)	0.73 (0.03)	0.086	1.55 (0.08)	1.51 (0.13)	0.823

All values were computed in an unadjusted model for all nondiabetes cases. Association to blood pressure is computed in all controls and diabetes cases without treatment of hypertension. Models on HOMA-%B and HOMA-IR are computed with log-transformed outcomes.

study populations revealed a *P* value of 0.0005 for association between *PTGES2* Arg298His SNP and type 2 diabetes. In EPIC, we also genotyped all htSNPs selected from a 20-kb gene region of *PTGES2* and a novel promoter SNP (g.-417G>T) detected in our sequencing approach. None of these showed a significant association with the disease. A common haplotype with the minor allele of Arg298His SNP showed evidence for association with type 2 diabetes similar to single SNP Arg298His. This finding substantiates the evidence that the Arg298His SNP within the *PTGES2* gene might be important for the association with type 2 diabetes.

It should be mentioned that many associations between polymorphisms and type 2 diabetes could not be verified in replication (32) or genome-wide association (33) studies. Therefore, although a replication cohort (KORA) was incorporated in our approach, the identified association between *PTGES2* Arg298His and the disease needs further replications in larger studies and functional analysis. Interestingly, we found a significant association between the *PTGES2* Arg298His and HOMA-%B as an indicator of basal insulin secretion. HOMA-IR values were also lower in His-carrier, but this difference was not significant. Both associations were found in controls but not cases. This could mean that the putative functionality of the polymorphism is apparent only under physiological conditions. A functional link between

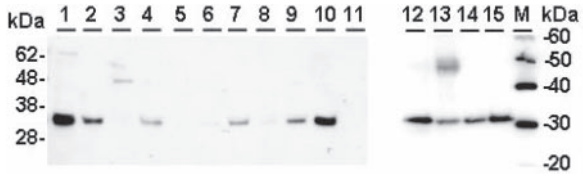

FIG. 1. Detection of the PTGES2 protein in different human tissues and cell lines by Western blot analysis. Protein extracts were separated by SDS-PAGE, blotted to polyvinyl difluoride membrane, and probed with a polyclonal anti-PTGES2 antibody. Lane 1, Huh7-cells (hepatocytes); lane 2, fully differentiated (13 d after confluency) SGBS cells (adipocytes); lane 3, undifferentiated (2 d after seeding) SGBS cells (preadipocytes); lane 4, partial differentiated (80% confluency) SGBS cells; lane 5, protein standard; lane 6, mitochondrial fraction from HepG2 cells (hepatocytes); lane 7, cytosolic fraction from HepG2 cells (hepatocytes); lane 8, mitochondrial fraction from fully differentiated (10 d after confluency) CaCo2-cells (intestine); lane 9, cytosolic fraction from fully differentiated (10 d after confluency) CaCo2 cells (intestine); lane 10, LNCaP cells (prostate); lane 11, HeLa cells (cervical); lane 12, muscle; lane 13, liver; lane 14 and 15, control lanes with 20 μg (lane 14) or 40 μg (lane 15) Huh-7 cells; lane 16, M (size marker in kDa). M, Protein molecular weight marker.

PGE_2 and β-cell dysfunction or impaired insulin secretion via the Akt (protein kinase B) pathway has been provided by cell (34–36) culture experiments *in vitro* and *ex vivo* as well as in animal studies and transgenic approaches (34–36). Assuming an expression of *PTGES2* in pancreatic β-cells, future studies of the *PTGES2* Arg298His SNP with respect to insulin secretion seem to be a promising functional approach. Because *PTGES2* is expressed in human skeletal muscle, liver, and differentiated adipocytes (Fig. 1), an influence of the Arg298His SNP on insulin sensitivity also has to be taken into account. As shown in cell culture experiments (37) and human studies, increasing concentrations of PGE_2 caused peripheral insulin resistance. A molecular mechanism for this effect has not been elucidated so far, but an alteration of insulin-stimulated phosphoinositide turnover by PGE_2 had been discussed earlier by Sandra and Marshall (37).

In addition to the investigated *PTGES2* transcript, three other transcripts are described in public databases (National Center for Biotechnology Information, Bethesda, MD). NM_198939 contains an additional internal exon that introduces a premature stop-codon resulting in an isoform with a distinct and shorter C terminus containing a glutaredoxin domain (NP_945177, 163 amino acids*). In silico* analysis revealed a localization of the identified SNP rs13283456 in the 3′UTR with no impact on the primary protein structure. NM_025072 encodes the 377 amino acids comprising membrane-associated PTGES2 (NP_079348), which was the focus of our study; NM_198940 and NM_198938 both encode an isoform of 186 amino acid residues (NP_945178 and NP_945176, respectively) with a shorter N terminus in comparison with NP_079348. In these three isoforms, rs13283456 causes the described amino acid exchange Arg→His, which is located in the glutathione-S-transferase domain. The change of a basic amino acid residue (Arg) with an acid ionization constant (pKa) value of 12.5 to a basic residue with a value of 6.0 (His) may cause a partial functional perturbation of the *PTGES2* His298 variant. This would result in lower PGE_2 production, which could influence β-cell function and/or insulin sensitivity.

In summary, we provide the first evidence from two independent German study populations that the His variant of the *PTGES2* Arg298His polymorphism is associated with reduced risk of type 2 diabetes. A hypothesis to explain this association is also provided.

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