Impact of the Hypoxia-Inducible Factor-1 α (*HIF1A*) Pro582Ser Polymorphism on Diabetes Nephropathy

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OBJECTIVE—Hypoxia plays a major pathogenic role in diabetic nephropathy (DN). We have investigated in this study the effect of hypoxia-inducible factor 1 α subunit (*HIF1A*) genetic polymorphisms on the development of DN.

RESEARCH DESIGN AND METHODS—In 1,165 American type 1 diabetic patients with and without DN selected from the Genetics of Kidneys in Diabetes (GoKinD) study, the *HIF1A* genetic polymorphisms were genotyped with TaqMan allelic discrimination. The regulation of HIF-1α in the kidneys of diabetic mice was appreciated by immunohistochemistry, and the effect *HIF1A* Pro582Ser polymorphism on HIF-1α sensitivity to glucose was evaluated in vitro.

RESULTS—We identified a protective association between *HIF1A* Pro582Ser polymorphism and DN in male subjects. We also provided mechanistic insights that HIF-1α is repressed in the medulla of diabetic mice despite hypoxia and that Pro582Ser polymorphism confers less sensitivity to the inhibitory effect of glucose during a hypoxic challenge.

CONCLUSIONS—The current study demonstrates for the first time that *HIF1A* Pro582Ser polymorphism has an effect on DN, possibly by conferring a relative resistance to the repressive effect of glucose on HIF-1α.

Diabetes Care 36:415–421, 2013

Diabetic nephropathy (DN) is the most common cause of end-stage renal disease (ESRD) and accounts for a high mortality rate in patients with diabetes (1). The role of genetic susceptibility in the development of DN has been known for many years. Identification of protective or risk genes may provide useful information about the pathogenesis of DN and permit the development of novel therapeutic approaches for this devastating complication (2).

Hypoxia has been proposed to have a central pathogenic mechanism for the development of DN (3). Hypoxia can be detected by magnetic resonance imaging (MRI) in the outer medulla of diabetic animals very early in the development of the disease, pointing out its primary pathogenic role (4). Renal hypoxia in diabetes is due to a reduction of oxygen delivery (5) and also to an increase in oxygen consumption at least partially secondary to an increase of respiratory uncoupling (6).

Hypoxia-inducible factor-1 (HIF-1) is the key mediator in cellular oxygen homeostasis that facilitates the adaptation to oxygen deprivation by regulating expression of gene products that are involved in cellular energy metabolism and glucose transport, angiogenesis, and erythropoiesis, among others (7). HIF-1 is a heterodimeric transcription factor composed of two subunits, HIF-1α and HIF-1β, both constitutively expressed in mammalian cells. Regulation of HIF-1 activity is critically dependent on the degradation of the HIF-1α subunit in normoxia. The molecular basis of its degradation is oxygen-dependent hydroxylation of at least one of the two proline residues (8) that makes HIF-1α accessible to the von Hippel-Lindau tumor-suppressor protein that acts as an E3 ubiquitin ligase and targets HIF-1α for proteasomal degradation (recently reviewed by Semenza [7]). Under hypoxic conditions, HIF-1 is transactivated and translocated to the nuclei where it binds to hypoxic responsive elements (HRE) and upregulates a series of genes essential for adaptation of the tissues to hypoxia (7). Diabetes has a complex repressive effect on the stabilization and transactivation of HIF-1α (9) precluding its optimal reaction to hypoxia, as recently reviewed (10).

HIF1A (for HIF-1α) genetic polymorphisms are associated with different responses to hypoxic injuries. For example, single nucleotide polymorphisms (SNPs) in the *HIF1A* gene are associated with different clinical outcomes (11) and collateral development (12) in subjects with coronary artery disease. Moreover, the prognosis after acute kidney injury is associated with a polymorphism in *HIF1A* (13). However, whether any *HIF1A* genetic polymorphisms are associated with DN is unknown.

In the current study, we conducted a genetic association study of the *HIF1A* gene in type 1 diabetic (T1D) patients with and without DN. Moreover, we investigated the modulation of renal HIF-1α in a mouse model of DN (*db/db*) and studied the functional relevance of *HIF1A* polymorphism (Pro582Ser) in the context of a combined challenge with hyperglycemia and hypoxia. We provide the first evidence indicating the effect of the SNPs in the *HIF1A* gene on the development of DN by its relative resistance to the repressive effect of hyperglycemia.

RESEARCH DESIGN AND METHODS

Diabetic patients with and without DN

The samples included 1,165 American T1D patients with and without DN selected

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Received 13 June 2012 and accepted 19 July 2012.
DOI: 10.2337/dc12-1125
A slide set summarizing this article is available online.

from the Genetics of Kidneys in Diabetes (GoKinD) study (11). All patients were of European descent, diagnosed before age 31 years, and treated with insulin within 1 year of diagnosis. The 571 T1D patients (case subjects) with DN (260 women, 311 men) had persistent proteinuria, defined by a urinary albumin-to-creatinine ratio ≤300 μg/mg in two of the last three measurements taken at least 1 month apart, or end stage renal disease (ESRD). Within case subjects, a subset of DN subjects had reached ESRD (*n* = 403; 188 women, 215 men) and formed the T1D-with-ESRD group. T1D patients without DN (control subjects, *n* = 594; 354 women, 240 men) had T1D for at least 15 years and normoalbuminuria, defined by an albumin-to-creatinine ratio <20 μg/mg in two of the last three measurements taken at least 1 month apart, without ever having been treated with ACE inhibitors or angiotensin-receptor blockers. They were not being treated with antihypertensive medication at the time of recruitment into the study. Clinical parameters of all subjects in the GoKinD population are summarized in Table 1.

All subjects were informed and consented to take part in the study. The procedures followed were in accordance with the Declaration of Helsinki II and approved by the local ethics committees. The material transfer agreements for the cohort of the GoKinD study were signed before experiments were performed. The study was approved by the local ethics committees.

SNPs selection and genotyping

Selection of SNPs in the *HIF1A* gene were done based on the information from the HapMap (http://www.hapmap.org/), dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), and previous genetic studies (13–15). Four nonsynonymous polymorphisms, including Pro582Ser, Thr588Ala, and Ile418Thr, in the HIF1A were included into the genotyping experiments. Genotyping experiments were carried out with TaqMan Allelic Discrimination assays by using ABI 7300 system (Applied Biosystems, Foster City, CA). For genotyping quality control, the subjects' samples were distributed randomly across plates with the case and control subjects per PCR plate. Negative controls (Universal-mixture blanks) were included on each plate. A subset of randomly selected samples representing ~20% of the study subjects was repeated.

Immunohistochemistry staining and evaluation

The hypoxia level and HIF-1α expression were evaluated in kidneys from C57BL/KsJm/*Leptdb* (*db/db*) mice and from their normoglycemic heterozygous littermates (16 weeks old) on formalin-fixed, paraffin-embedded sections (5 μm). Both stainings were performed after the slides were deparaffinized and rehydrated.

For HIF-1α staining, the slides were pretreated by microwave heating (800 W for 20 min) in citrate buffer, followed by endogenous peroxidase blocking (20 min at room temperature) and blocking with normal goat serum. The incubation with the primary antibody (NB: 100–123, Novus Biologicals; 1:100) was performed overnight at 4°C, followed by exposure to the secondary biotinylated antibody (goat anti-mouse) for 10 min at room temperature. The reaction was developed using an ABC kit (Vector Laboratories) and subsequent DAB treatment. Matched IgG isotype controls were included.

Hypoxia was evaluated using the Hypoxiprobe kit (Natural Pharmacia International, Burlington, MA) following the manufacturer's instructions. The extent of hypoxic areas and of HIF-1α positive areas was quantified by computer-assisted image analysis and was expressed as the percentage of positive stained area versus total tissue area.

Plasmid constructs

Plasmid encoding FLAG-fused mouse HIF-1α in which Proline 583 (which is the mouse equivalent of the human Proline 582) was mutated to serine (pFLAG/mHIF-1α [P/S]) was generated using QuikChange site-directed mutagenesis kit (Stratagene) according to the manufacturer's instructions, and positive mutants were screened by sequencing using DYEnamic sequencing kit (Amersham Biosciences). The *Renilla* luciferase reporter vector (pRL-TK) was obtained from Promega. Plasmids encoding FLAG-fused wild-type mouse HIF-1α (pFLAG/mHIF-1α) and hypoxia response element (HRE)-driven firefly luciferase reporter (pT81/HRE-luc) have been described previously (14,15).

Cell culture

Human embryonic kidney (HEK) 293A cells were maintained in a 1:1 mixture of DMEM and F-12 medium. Media were supplemented with FCS (10%), penicillin (50 IU/mL), and streptomycin sulfate (50 μg/mL). Medium and other products

Table 1—*Clinical characteristics of the GoKinD population*

	T1D with DN			T1D without DN		
	All *N* = 571	Female *n* = 260	Male *n* = 311	All *N* = 594	Female *n* = 354	Male *n* = 240
Age (years)	44 ± 6	44 ± 7	45 ± 6	40 ± 8	40 ± 9	40 ± 8
Duration (years)	32 ± 8	32 ± 8	32 ± 8	26 ± 8	26 ± 8	26 ± 8
HbA_{1c} (%)	7.4 ± 1.9	7.4 ± 2.1	7.4 ± 1.7	7.5 ± 1.1	7.5 ± 1.1	7.4 ± 1.1
BMI (kg/m^2)	25.7 ± 5.3	25.2 ± 5.7	26.1 ± 4.9	26.0 ± 4.4	25.7 ± 4.7	26.6 ± 3.7
Creatinine (mg/dL)	2.1 ± 1.9	1.9 ± 1.6	2.3 ± 2.0	0.9 ± 0.2	0.8 ± 0.1	1.0 ± 0.1
Cystatin (mg/L)	2.2 ± 1.7	2.2 ± 1.6	2.4 ± 1.7	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
Cholesterol (mg/dL)	186.3 ± 45.9	190.3 ± 47.3	183.0 ± 44.6	185.0 ± 31.3	188.7 ± 30.9	179.7 ± 31.2
HDL (mg/dL)	53.6 ± 17.3	59.3 ± 18.2	48.9 ± 15.1	59.1 ± 15.9	64.8 ± 15.5	51.0 ± 12.4
Blood pressure (mmHg)						
Systolic	131 ± 19	130 ± 20	133 ± 18	118 ± 12	116 ± 12	122 ± 12
Diastolic	74 ± 11	72 ± 11	75 ± 11	71 ± 8	70 ± 7	74 ± 8

All data are means ± SD.

for cell culture were purchased from Invitrogen. Cells were cultured at 37°C under normoxic (21% O_2) or hypoxic (1% O_2) conditions.

Transient transfections

Transient transfections in 293A cells were performed using Lipofectamine (Invitrogen) according to the manufacturer's instructions. One microgram of plasmids pFLAG/mHIF-1α or pFLAG/mHIF-1α (P/S) were transfected into 293A cells to detect their expression level. In the dual-luciferase reporter assay (Promega), 293A cells were cotransfected with pT81/HRE-luc (300 ng), pRL-TK (30 ng), pFLAG/mHIF-1α, or pFLAG/mHIF-1α (P/S; 100 ng), and pCMX empty vector (570 ng). At 16 h after transfection, the cells were cultured in media containing different concentrations of glucose and exposed to normoxia (21% O_2) or hypoxia (1% O_2), as indicated. The cells were lysed in passive lysis buffer (Promega) 48 h later, and the luciferase activity was measured according to the manufacturer's instructions. The relative luciferase activity was calculated as the HRE-driven firefly luciferase activity-to-*Renilla* luciferase activity.

Protein extraction and immunoblotting assays

Protein extraction and immunoblotting assays were performed as described before (16). Briefly, cells were washed with PBS, collected by centrifugation, and lysed by sonication in a buffer containing 50 mmol/L Tris-HCl (pH 7.4), 180 mmol/L NaCl, 0.2% NP-40, 20% glycerol, 0.5 mmol/L phenylmethylsulfonyl fluoride, 5 mmol/L β-mercaptoethanol, and protease inhibitor mix (Complete-Mini; Roche Biochemicals). The lysates were then cleared by centrifugation for 30 min at 14,000 rpm at 4°C. The whole-cell extracts were separated by SDS-PAGE and blotted onto nitrocellulose membranes. Blocking was performed in TBS buffer (50 mmol/L Tris [pH 7.4], 150 mmol/L NaCl) containing 5% nonfat milk, followed by incubation with anti-FLAG M2 (F3165, Sigma) or anti–β-actin (ab6276, Abcam) antibodies in TBS buffer containing 1% nonfat milk. After several washes with TBS buffer containing 0.5% Tween-20, the membranes were incubated with anti-mouse or anti-rabbit IgG-horseradish peroxidase conjugate (Amersham Biosciences) in TBS buffer containing 1% nonfat milk. After several washes, proteins were visualized using enhanced chemiluminescence (Amersham Biosciences) according to the manufacturer's recommendations.

Statistical analyses

To evaluate the genotype distribution, deviation for genotyped SNP from Hardy-Weinberg equilibrium (HWE) was assessed using the Pearson χ^2 test. Case and control subject analyses were performed initially in T1D patients with DN (case subjects) and without DN (control subjects). To assess for the association with ESRD, a subset group (T1D with ESRD) composed of case subjects was contrasted with control subjects in succession. Odds ratios (OR) and 95% CI were calculated to test the relative risk for association. Statistical powers were calculated using PS 2.1.31 software (PowerSampleSize, http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize). Tests for association between genotypes and quantitative traits were performed by using ANOVA for normally distributed traits. Kruskal-Wallis analysis of ranks was alternatively used for traits with nonnormal distributions. Normal probability plots were created and parameter distributions were transformed to natural logarithm when needed to improve the skewness and for obtaining a normal distribution before performing statistical analysis of the relevant phenotypes. Data are given as the means ± SD. The Student *t* test was used to analyze the difference between two samples in gene expression and biological experiments. A value of $P < 0.05$ was considered significant. All data were analyzed using IBM SPSS Statistics 20 software (IBM Corporation, Armonk, NY).

Differences between immunohistochemistry results were computed using two-tailed *t* test analysis between groups, and the differences between reporter gene results were computed using one-way ANOVA with Bonferroni post hoc test. $P < 0.05$ was considered statistically significant.

RESULTS

Genetic association of *HIF1A* with DN

We began with a genetic association study of three SNPs (Pro582Ser, Thr588Ala, and Ile418Thr) selected from the *HIF1A* gene in the GoKinD population. Genotype distribution of all studied SNPs in this population was satisfied in the HWE assumption. Minor allele frequencies of all studied SNPs are summarized in Table 2.

We analyzed the possible association of *HIF1A* polymorphisms with DN in the GoKinD population. Data indicated that there was an association of Pro582Ser polymorphism in the *HIF1A* gene (OR 0.758 [95% CI 0.590–0.973], $P = 0.029$; Table 3) but no significant association with DN of the other two studied polymorphisms was found (data not shown). We further analyzed sex stratification for this association and found that the association of this Pro582Ser polymorphism in the *HIF1A* gene with DN (0.613 [0.422–0.888], $P = 0.009$; Table 3) and ESRD (0.611 [0.404–0.924], $P = 0.018$; Table 3) was maintained in men but not in women.

HIF regulation in DN

To understand the biological relevance of the above genetic association, we investigated the modulation of HIF in the kidney of *db/db* mice, a well-established model of DN (17). The expression of HIF-1α was analyzed in the context of the hypoxia level (as evaluated by pimonidazole staining), which is an established pathogenic factor for DN and the principal regulator of HIF. Indeed, we noticed an increase of the hypoxia in the medulla of the diabetic animals, as evaluated by pimonidazole adduct formation (Fig. 1*A*). However, the HIF-1 levels did not mirror the oxygen levels (Fig. 1*B*), being even inappropriately repressed in the medulla of the diabetic animals compared with the

Table 2—*Minor allele frequencies of the* HIF1A *polymorphisms in the GoKinD population*

Gene symbol	dbSNP rsID	SNP type	Chromosome position*	MAF all (female/male)
HIF1A	rs11549465	Y = C/T Pro582Ser	Chr14, 42257827	T 0.122 (0.128/0.115)
	rs11549467	R = A/G Ala588Thr	Chr14, 42257845	A 0.016 (0.016/0.015)
	rs41508050	Y = C/T Ile418Thr	Chr14, 42255074	T 0.028 (0.032/0.024)

MAF, minor allele frequency; R, adenine or guanine; S, cytosine or guanine; Y, cytosine or thymine. Y, R, and S are International Union of Pure and Applied Chemistry (IUPAC) codes used in FASTA sequences at the SNP position. *References: NW_925561.1 (HIF1A) and NW_924884.1 (HIF1AN).

Table 3—*Associations of the* **HIF1A** *Pro582Ser polymorphism with DN and ESRD in the GoKinD population*

T1D group	Sex	Genotype, *N*			MAF	OR (95% CI)	*P*
		CC	CT	TT			
With ESRD	All	321	79	3	0.105	0.747 (0.565–0.987)	0.039
	Female	145	41	2	0.120	0.899 (0.615–1.315)	0.583
	Male	176	38	1	0.093	0.611 (0.404–0.924)	0.018
With DN	All	453	114	4	0.107	0.758 (0.590–0.973)	0.029
	Female	199	58	3	0.123	0.928 (0.660–1.305)	0.667
	Male	254	56	1	0.093	0.613 (0.422–0.888)	0.009
Without DN	All	439	148	7	0.136		
	Female	265	85	4	0.131		
	Male	174	63	3	0.144		

MAF, minor allele frequency; *N*, number of genotyped samples. *P* values and ORs are tested by comparison analyses for T1D with DN or T1D with ESRD vs. T1D without DN. CC, CT, and TT are the genotypes of *HIF1A* Pro582Ser polymorphism.

medulla of the normoglycemic heterozygote animals (Fig. 1*C*).

Biological effects of *HIF1A* Pro582Ser polymorphism

Having in account the pathological repression of HIF in diabetic kidneys, we have further investigated the potential functional relevance of Pro582Ser polymorphism in the reaction of the cells to hypoxia and hyperglycemia that are essential pathogenic mechanisms for DN. By using FLAG-tagged HIF-1α constructs, wild-type or mutated at the equivalent mouse proline (Pro/Ser), we first followed the effect of the polymorphism on HIF stability. Wild-type HIF-1α and HIF-1α (Pro/Ser) have a similar expression level at normoxia and hypoxia, suggesting that the polymorphism is not crucial for HIF stabilization in hypoxia (Fig. 2*A*). We next investigated if hyperglycemia affects the stability of HIF-1α (P/S) as it does for wild-type HIF-1α (9,16) by exposing the transfected cells to different glucose concentrations and found that indeed hyperglycemia destabilizes HIF-1α (P/S) as well (Fig. 2*B*).

We further assessed the relevance of the polymorphism on HIF-1α transcriptional activation using HRE-reporter gene in HEK 293A cells cultured in different concentrations of glucose in normoxia or hypoxia (Fig. 2*C*). Wild-type HIF-1α and HIF-1α (P/S) both activate the HRE-reporter gene at the similar level in normal glucose concentration (5.5 mmol/L) in normoxia or when activated by hypoxia. In hypoxia, however, HIF-1α (P/S) showed significantly higher activity than wild-type HIF-1α when the cells were cultured in high glucose concentrations. In normoxia, no difference was noted between wild-type HIF-1α and HIF-1α (P/S) when the cells were exposed to high glucose concentrations. Results indicated that Pro582Ser polymorphism confers relative resistance to the repressive effect of

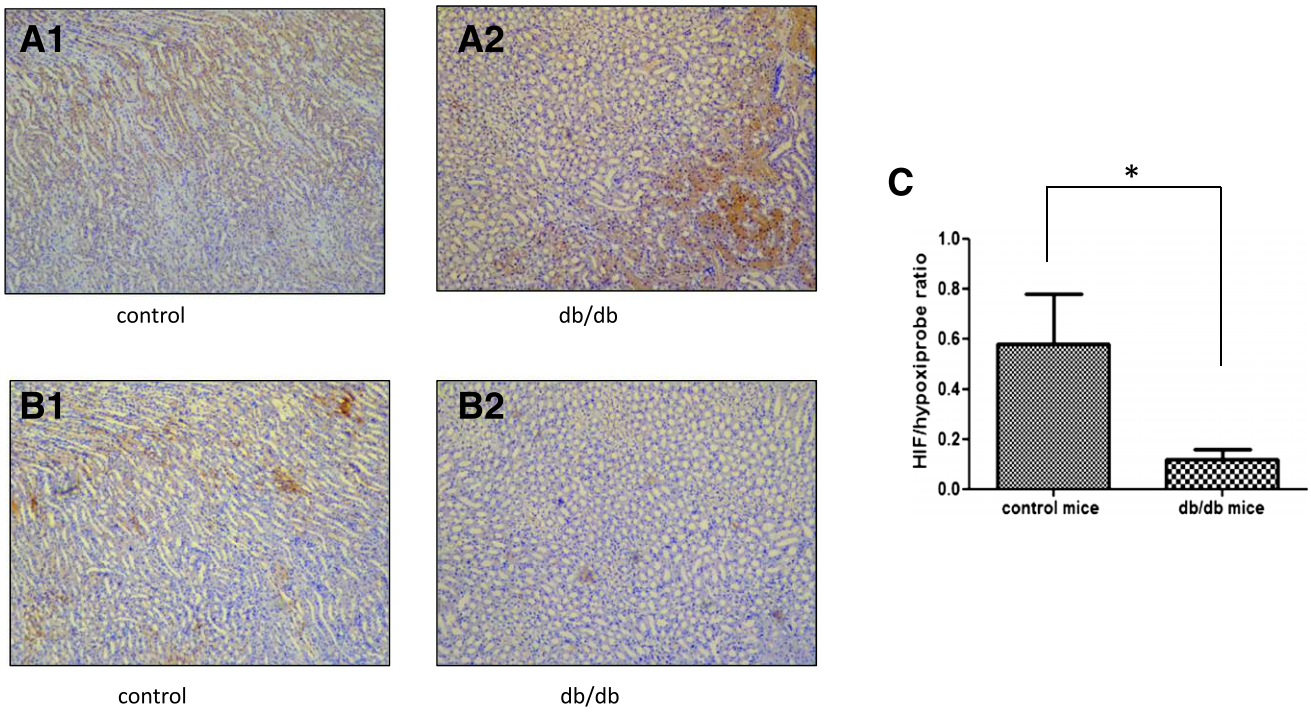

Figure 1—*HIF-1α is repressed in diabetic kidneys medulla despite hypoxia.* A: *The kidney medulla of the diabetic animals* (db/db) (A2) *is more hypoxic than in normoglycemic heterozygote control mice* (A1)*, as evaluated by pimonidazole adduct formation.* B: *HIF-1α expression in the medulla of the diabetic mice* (db/db) (B2) *is lower than in normoglycemic control mice* (B1). C: *The ratio between areas positive for HIF-1α and hypoxic areas (evaluated by pimonidazole) is lower in the medulla of diabetic animals compared with normoglycemic control mice.**$P < 0.05$. *(A high-quality digital representation of this figure is available in the online issue.)*

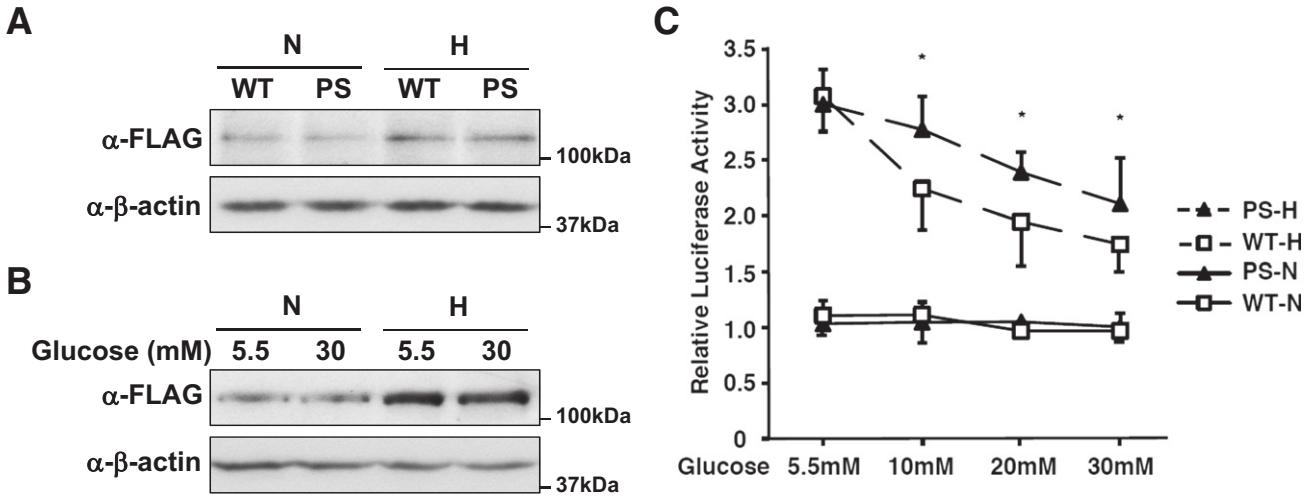

Figure 2—*Effects of* HIF1A *Pro582Ser polymorphism in glucose levels.* A*: Wild-type HIF-1α and HIF-1α (P/S) have similar expression level in normoxia and hypoxia. HEK293A cells were transfected with pFLAG/mHIF-1α (wild-type [WT]) or pFLAG/mHIF-1α (P/S), and were exposed to normoxia (N) or hypoxia (H) for 6 h before harvest. The expression of HIF-1α and mutant was detected by immunoblotting using anti-FLAG antibody.* B*: HIF-1α (P/S) protein is downregulated by 30 mmol/L glucose. HEK293A cells were transfected with pFLAG/mHIF-1α (P/S). The cells were then cultured in media containing 5.5 or 30 mmol/L glucose for 48 h and were exposed to normoxia (N) or hypoxia (H) for 6 h before harvest. The expression of HIF-1α (P/S) was detected by immunoblotting using anti-FLAG antibody.* C*: HIF-1α (P/S) is more active than wild-type HIF-1α under conditions of combined hyperglycemia and hypoxia. In a dual-luciferase reporter assay, HEK293A cells were transfected with pFLAG/mHIF-1α or pFLAG/mHIF-1α (P/S) together with reporter plasmids. The cells were then cultured in media containing 5.5, 10, 20, and 30 mmol/L glucose and exposed to normoxia (N) or hypoxia (H) for 48 h. The activity of wild-type HIF-1α (WT) and HIF-1α (P/S) is presented as mean ± SD. *P < 0.05 comparing the activity of WT and P/S at indicated concentration of glucose under hypoxic conditions.*

hyperglycemia on HIF-1α when challenged by hypoxia.

CONCLUSIONS—We have identified for the first time an association between DN and the *HIF1A* Pro582Ser polymorphism. Moreover, we showed that this functional variant has a specific potential mechanistic role by conferring a relative resistance for the kidney to hypoxic insult in the presence of hyperglycemia.

Hypoxia has a central pathogenic mechanism for DN being recorded early during evolution of the disease, especially in the medulla (18). HIF plays an essential role in modulating the reaction of the tissues in hypoxia (7). Its function, however, is repressed in diabetes (9) and contributes to an inappropriate reaction of the tissues to hypoxic injury (10). We observed a similar inappropriate reaction of HIF to hypoxia (Fig. 1) in the medulla of *db/db* mouse kidney, an established animal model for DN (17). The HIF repression in diabetic kidney (19) is pathogenically relevant because HIF induction by $CoCl_2$ treatment reduces the progression of DN (20). It is therefore probably with minor functional consequences the paradoxical activation of HIF observed in diabetic kidneys in isolated cells (i.e., mesangial cells [21] and podocytes [22]).

In this context, the association between Pro582Ser polymorphism of HIF-1α and the risk that DN will develop is not surprising. The same Pro582Ser polymorphism is associated with stable exertional angina but not with acute myocardial infarction as the first manifestation of coronary arterial disease (23), conferring the same protective phenotype that we found in DN. It is therefore expected that the same polymorphism confers a worse prognosis in clinical situations where HIF has deleterious effects, as in cancer (24). A worse prognosis is conferred by Pro582Ser after acute kidney injury (13), even though it is unclear what role HIF-1 plays in this clinical situation. An association of this polymorphism to type 2 diabetes was reported in a Japanese population (25), which is not surprising because overexpression of HIF-1α in β-cells has deleterious effect on glucose tolerance (26,27).

The protective effect of Pro582Ser for DN was observed just in male subjects. A sexual dimorphism for this functional SNP has been also reported for cancer (24). Functional studies show that testosterone induces HIF function in rats, with a protective effect in myocardial infarction (28), whereas estrogens have repressive effects for HIF in adipose tissue (29), which adds a possible mechanistic explanation to our results. We have, moreover, demonstrated that Pro582Ser confers a functional advantage against the repressive effect of glucose on HIF function. Two Proline residues (Pro402 and Pro564) are essential for HIF-1α degradation in normoxia (8,30). However, Pro582 has no essential role in HIF-1α stability because its mutation with serine does not affect HIF-1α levels, neither in normoxia nor hypoxia (Fig. 2*A*), which is in perfect agreement with previous observations (31). Moreover Pro582Ser does not have a central role in the destabilizing effect of glucose on HIF-1α (9) because we observed a clear destabilization of the Pro582Ser construct in hyperglycemia (Fig. 2*B*). However, Pro582Ser has a profound effect for the sensitivity of HIF-1α to hyperglycemia at the transactivation levels (Fig. 2*C*), which offers the functional explanation for its protective effect for DN. Some other residues (L556A/L558A and M560/L561) (32), including Pro582 (33), were identified to modulate the transactivation but not stability of HIF in hypoxia in other experimental conditions. However, the special sensitivity to

glucose of HIF (P/S) transactivation in hypoxia is unique to our knowledge, suggesting a specific role in diabetes.

In conclusion, our study provides the first evidence for a protective polymorphism of the *HIF1A* gene for DN and provides the mechanistic explanation for it. It is of high interest to investigate the potential association of this polymorphism with other clinical conditions in which diabetes has deleterious effects on outcome at least partially through HIF-1 repression (i.e., myocardial infarction, diabetic nonhealing foot ulcers, etc.).

Acknowledgments—The GoKinD collection was supported by the Juvenile Diabetes Research Foundation in collaboration with the Joslin Diabetes Center and George Washington University, and by the United States Centers for Diabetes Control and Prevention. The study was supported by grants from Family Erling-Persson Foundation, Swedish Research Council, Stig and Gunborg Westmans Foundation, von Kantzow Foundation, and Stockholm County Council.

No potential conflicts of interest relevant to this article were reported.

H.F.G. and S.-B.C. analyzed data, designed the research, and wrote the manuscript. X.Z. and I.R.B. performed research and analyzed data. N.A.S., T.G., V.G.S., and E.F.L. performed research. K.B. reviewed the manuscript. S.-B.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

The authors thank all subjects in the GoKinD population for their participation and thank Stina Lindberg, Department of Molecular Medicine and Surgery, for excellent technical and laboratory assistance.

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