Growth-Differentiation Factor 15 Predicts Worsening of Albuminuria in Patients With Type 2 Diabetes

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OBJECTIVE—Development of micro- or macroalbuminuria is associated with increased risk of cardiorenal complications, particularly in diabetes. For prevention of transition to micro- or macroalbuminuria, more accurate prediction markers on top of classical risk markers are needed. We studied a promising new marker, growth-differentiation factor (GDF)-15, to predict transition to increasing stage of albuminuria in type 2 diabetes mellitus (T2DM). In addition, we looked at the GDF-15 potential in nondiabetic subjects with hypertension (HT).

RESEARCH DESIGN AND METHODS—Case and control subjects were selected from the PREVEND cohort, a large (n = 8,592), prospective general population study on the natural course of albuminuria, with >10 years of follow-up and repeated albuminuria measurements. We found 24 T2DM and 50 HT case subjects transitioning from normo- to macroalbuminuria and 9 T2DM and 25 HT case subjects transitioning from micro- to macroalbuminuria (average follow-up 2.8 years). Control subjects with stable albuminuria were pair matched for age, sex, albuminuria status, and diabetes duration. GDF-15 was measured in samples prior to albuminuria transition.

RESULTS—Prior to transition, GDF-15 was significantly higher in case subjects with T2DM than in control subjects (median [IQR] 1,288 pg/mL [885–1,546] vs. 948 pg/mL [660–1,016], $P < 0.001$). The odds ratio for transition in albuminuria increased significantly per SD of GDF-15 (2.9 [95% CI 1.1–7.5], $P = 0.03$). GDF-15 also improved prediction of albuminuria transition, with significant increases in C statistic (from 0.87 to 0.92, $P = 0.03$) and integrated discrimination improvement (0.148, $P = 0.001$). In HT, GDF-15 was also independently associated with transition in albuminuria stage (2.0 [1.1–3.5], $P = 0.02$) and improved prediction significantly.

CONCLUSIONS—We identified GDF-15 as a clinically valuable marker for predicting transition in albuminuria stage in T2DM beyond conventional risk markers. These findings were confirmed in nondiabetic HT subjects.

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The prevalence of chronic kidney disease is increasing and has become a major public health challenge (1). This increase in chronic kidney disease is largely due to the rapidly expanding epidemic of type 2 diabetes mellitus (T2DM) leading to diabetic nephropathy and ultimately end-stage renal disease (2). Transition to increasing stages of albuminuria (i.e., normo- to microalbuminuria and micro- to macroalbuminuria) is considered a hallmark of progression of renal disease in diabetes (3). However, once disease has transitioned from normo- to microalbuminuria or to macroalbuminuria, regression of disease is very difficult to achieve. Indeed, recent trials in normo-, micro-, and macroalbuminuric diabetic subjects showed that early intervention (in normoalbuminuric stage) is more effective than late intervention (4). Early markers that detect those who have an increased risk for developing micro- or macroalbuminuria could thus help to reduce the number of patients at renal risk through selective and appropriate treatment of such patients (5,6).

Many risk factors have been linked to transition from normo- to micro- and from micro- to macroalbuminuria, such as hyperglycemia, hypercholesterolemia, and hypertension (7). However, accurate risk stratification remains challenging. Novel biomarkers may help to improve the identification of subjects at risk, as well as improve insight into the underlying pathophysiology of the development of micro- or macroalbuminuria. Whereas several promising novel biomarkers have been described in the literature, this had not led to improved risk stratification in T2DM (8).

The lack of well-designed prospective studies that first stored samples of individuals for novel risk marker analyses and then followed the course of albuminuria over time may explain the paucity of knowledge on the prognostic value of novel biomarkers to improve risk stratification. We performed a nested case-control study in the large general population cohort Prevention of REnal and Vascular End-stage Disease (PREVEND) to investigate novel biomarkers that may precede and predict the transition in albuminuria (9).

Growth differentiation factor (GDF)-15, a member of the transforming growth factor-β family, is a promising novel biomarker that has been implicated as a predictor for cardiovascular and all-cause mortality (10–12). Interestingly, it was also associated with renal outcome and a faster decline of estimated glomerular filtration rate (eGFR) as well as mortality in type 1 diabetic patients with macroalbuminuria (13). It is unclear whether these findings regarding renal outcome are also applicable to patients with type 2 diabetes. In the current study, we investigated whether circulating GDF-15 levels precede and predict the development of micro- or macroalbuminuria in type 2 diabetic patients. To test whether this is specific to diabetes, we performed a replication study to assess the predictive value of GDF-15 in nondiabetic hypertensive patients.

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RESEARCH DESIGN AND METHODS

—The current study was performed as a nested case-control study in subjects participating in the PREVEND study. This prospective community-based cohort study on the natural course of urinary albumin excretion (UAE) with serial follow-up measurements was initiated in 1997. Details of the study protocol have previously been published (9). In short, all inhabitants of the city of Groningen aged 28–75 years were sent a questionnaire and a vial to collect a first-morning void urine sample. Of these individuals, 40,856 responded (47.8%). From these individuals, a cohort consisting of 8,592 subjects was selected (the PREVEND cohort). In this ongoing study, participants are invited to visit an outpatient clinic for detailed medical examination at intervals of ±3 years. At each screening round, participants fill out questionnaires on demographics, medical history, and drug use. Information on drug use is completed with data from community pharmacies, including information on class of antihypertensive medication (ACE inhibitor [ACEi]/angiotensin-2 receptor blocker [ARB]). At the study visits, participants deliver two 24-h urine collections, blood pressure is measured, anthropometrical measurements are performed, and fasting blood samples are taken.

The PREVEND study was approved by the institutional ethics review board and was conducted in accordance with the guidelines of the Declaration of Helsinki. All participants provided written informed consent.

Definitions

Normoalbuminuria was defined as UAE <30 mg/24 h, microalbuminuria as UAE 30–299 mg/24 h, and macroalbuminuria as UAE ≥300 mg/24 h. Albuminuria status was based on the average of two consecutive measurements in 24-h urine collections. Transition in albuminuria was defined as a transition from normo- to micro- or from micro- to macroalbuminuria with at least 30% increase in UAE from baseline between two consecutive study visits. T2DM was defined as the use of oral antidiabetes treatment (self-reported or by information retrieved from the regional pharmacy database), a fasting plasma glucose >7.0 mmol/L (126 mg/dL), or nonfasting plasma glucose >11.1 mmol/L (>200 mg/dL). Hypertension (HT) was defined as the use of antihypertensive treatment or a systolic/diastolic blood pressure >140/90 mmHg.

Selection of case and control subjects

For the current study, we selected patients with T2DM and transition from normo- to microalbuminuria or from micro- to macroalbuminuria. As control subjects, we selected T2DM patients who had persistent normoalbuminuria or microalbuminuria throughout the same time interval as case subjects (Fig. 1*A*). As a secondary cohort, we selected similar case and control subjects out of all nondiabetic patients with HT (Fig. 1*B*). Control subjects were matched to the case subjects 1:1 based on diabetes/nondiabetes, age, sex, baseline normo- or microalbuminuria status, and, if applicable, duration of diabetes. For each subject that showed transition in albuminuria, a matched control subject was selected that most optimally resembled the case subject on these combined parameters. Plasma samples of these patients were used from the visit prior to the transition from normo- to microalbuminuria or from micro- to macroalbuminuria (baseline). The use of agents intervening in the

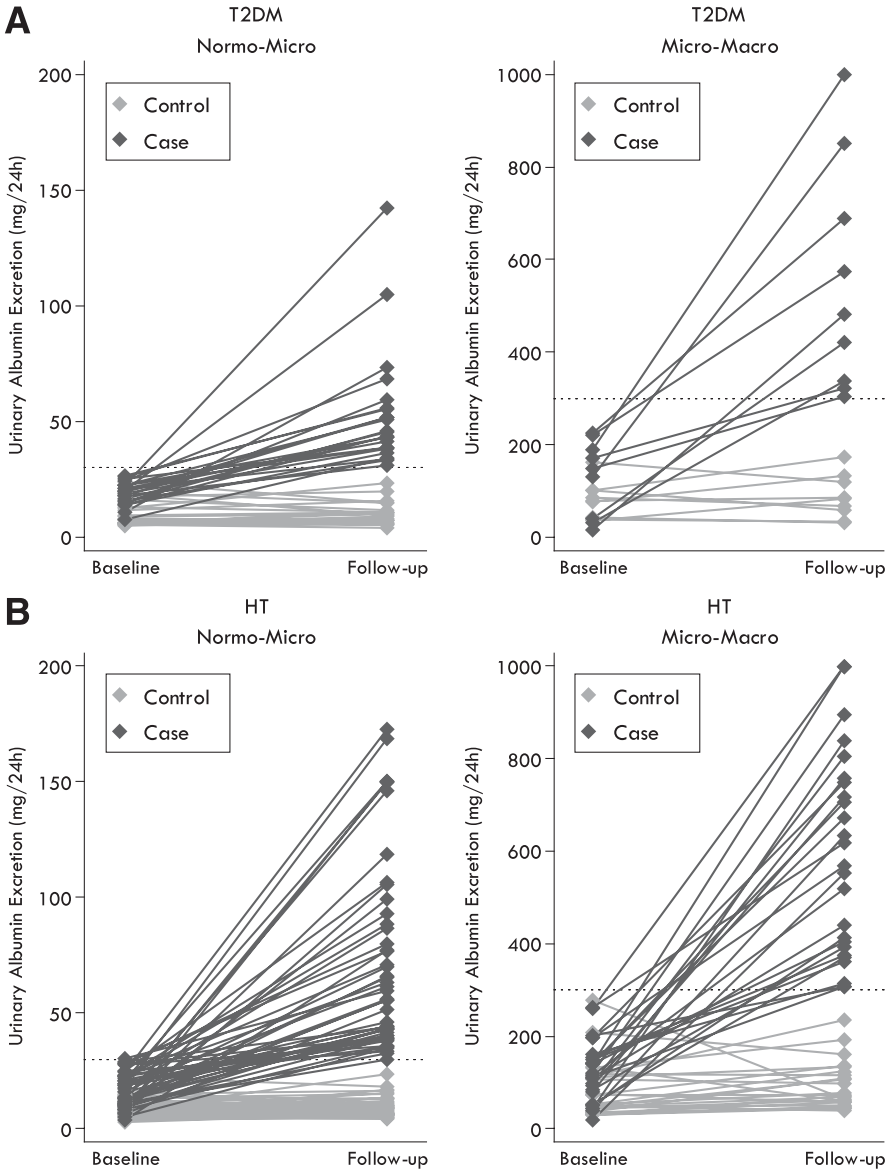

Figure 1—*Individual courses of UAE in progressors and nonprogressors in albuminuria (case and control subjects). Individual course of UAE during follow-up for case (black) and control (gray) subjects in patients with T2DM* (A) *or HT* (B) *stratified for albuminuria status at baseline (left: normoalbuminuria; right: microalbuminuria).*

renin-angiotensin-aldosterone system (ACEi/ARB) was allowed, but the type of drug and their dose had to remain stable during the study period.

Of the 8,592 study participants, 318 had T2DM, and 33 of these subjects had unambiguous transition in albuminuria and available samples of sufficient quality. HT (without diabetes) was present in 1,178 participants. Of these participants, 75 subjects had unambiguous transition in albuminuria and available samples of sufficient quality.

Measurements

Plasma samples were stored at −80°C, and all samples underwent one freeze-thaw cycle. Measurements were performed blinded and in duplicate. GDF-15 was measured with a novel precommercial assay based on the Eclia principle (Roche Diagnostics) with a lower limit of detection (LLD) of 200 pg/mL and intraindividual coefficient of variation (CV) of 6.7–9.2%.

UAE is given as the mean of the two 24-h urinary excretions. Blood pressure was measured twice, in the supine position, every min for 10 min with an automatic device (Dinamap XLModel 9300; Johnson & Johnson Medical, Tampa, FL). eGFR was estimated with the Modification of Diet in Renal Disease study equation, using sex, age, race, and serum creatinine (14).

Urinary albumin concentration was determined by nephelometry (Siemens, Munich, Germany). Concentrations of total cholesterol and plasma glucose were measured using standard methods. Serum creatinine was measured by dry chemistry (Eastman Kodak, Rochester, New York), with intra-assay CV of 0.9% and interassay CV of 2.9%.

Statistical analysis

Analyses were performed using STATA version 11.2 (StataCorp, College Station, Texas). A study with 50 patients will provide at least 80% power to detect an odds ratio of 1.5 assuming a type 1 error of 5% and no residual confounding after matching case and control subjects (www.bioconductor.org). Variables with normal distribution are given as means ± SD and variables with skewed distribution as median (interquartile range [IQR]). Variables with skewed distribution were log transformed for analyses. Graphical methods were used to ascertain normalization of the distribution after transformation. Differences between the case and control subjects were tested with paired-sample *t* test for continuous variables and χ^2 test on paired proportions for categorical variables. Differences between nonpaired groups were tested with independent-sample *t* test for continuous variables and χ^2 test for categorical variables.

To investigate the association between the levels of the markers and transition in albuminuria, we used conditional logistic regression because of the paired study design. In multivariable analyses, we adjusted for differences in baseline UAE and eGFR between case and control subjects, as these two markers are important risk factors for transition in albuminuria stage. We added several sensitivity analyses, adjusting for other important risk markers for transition in albuminuria, and combining patients with T2DM and patients with HT. We tested for interaction between patients who made a transition from normo- to microalbuminuria and those who made a transition from micro- to macroalbuminuria by adding an interaction term for baseline albuminuria status and GDF-15 in the model.

To assess whether the markers improved risk prediction and discrimination, we determined the *C* statistic and integrated discrimination improvement (IDI). We calculated the *C* statistic (discriminatory ability) based on the most important established risk factors (UAE and eGFR) and compared those to *C* statistics after addition of GDF-15 to the established model. Differences in *C* statistic were tested with χ^2 test.

In addition to the *C* statistic, we calculated the IDI, another measure of discrimination (15). The IDI is the difference in discrimination slopes between case and control subjects before and after the addition of the biomarker(s) to the model. It assesses the improvement in average sensitivity without sacrificing average specificity (16). Calculation of the IDI is done by computing average predicted probabilities of the event in case and control subjects in models with and without the biomarker(s) and subtracting the values from case and control subjects from each other. The increase in difference between case and control subjects after addition of the biomarker(s) is the integrated reclassification improvement. The IDI is often more sensitive than the rather conservative *C* statistic because when several highly predictive markers are in the model, enormous odds ratios are required to meaningfully increase the *C* statistic (17). For all analyses, two-sided *P* values <0.05 were considered statistically significant.

RESULTS—In total, 33 case subjects with T2DM and transition from normo- to microalbuminuria or from micro- to macroalbuminuria were identified. These case subjects were pair matched to 33 control subjects with T2DM and stable albuminuria.

The baseline characteristics are presented in Table 1. Mean age of case subjects was 62.7 years, 25 (75.8%) patients were male, and the median UAE rate was 18 g/24 h (IQR 9–44). Median follow-up was 2.7 years (2.2–3.8). Case subjects had a significantly higher baseline UAE than control subjects, more frequently received lipid-lowering treatment, and more often received ACEi/ARB treatment compared with matched control subjects. All other parameters were similar. Median follow-up time was 2.7 years (2.2–4.0).

Change in albuminuria concentrations during follow-up, according to the selection of case and control subjects, is shown in Fig. 1*A*. Patients with transition in albuminuria (case subjects) had a median increase in albuminuria of 31 mg/24 h (164%) compared with 0 mg/24 h (0%) in control subjects.

GDF-15 in T2DM patients

The mean concentration of GDF-15 in case subjects and for control subjects, prior to transition in albuminuria, is presented in Fig. 2*A*. In Fig. 2*B*, these concentrations are presented separately for transition from normo- to micro- and from micro- to macroalbuminuria. GDF-15 concentrations were significantly higher in case vs. control subjects (median 1,288 pg/mL [IQR 885–1,546] vs. 948 pg/mL [660–1,016], $P < 0.001$). The concentrations were lower in normoalbuminuric case and control subjects than in microalbuminuric case and control subjects (910 pg/mL [737–1,162] vs. 1,008 pg/mL [763–1,470], $P = 0.03$).

The odd ratio for transition in albuminuria was 3.58 [95% CI 1.51–8.47] (per SD increment in GDF-15) (Table 2) and 2.87 [1.10–7.53] after adjustment for baseline albuminuria and eGFR. There was no statistical significant difference between the odds for transition from normo- to microalbuminuria and from micro- to macroalbuminuria with each SD increment in GDF-15 (*P* for interaction = 0.55). GDF-15 significantly improved the *C* statistic on top of a baseline model

Table 1—*Baseline characteristics for patients with T2DM and nondiabetic patients with HT*

	T2DM		HT	
Patient characteristic	Case subjects	Control subjects	Case subjects	Control subjects
n	33	33	75	75
Age (years)	62.7 ± 9.2	62.8 ± 9.6	66.3 ± 9.8	65.6 ± 8.7
Male sex	25 (75.8)	25 (75.8)	53 (70.7)	53 (70.7)
Race				
Caucasian	30 (90.9)	31 (93.9)	71 (94.7)	73 (97.3)
Other	3 (9.1)	2 (6.1)	4 (5.3)	2 (2.7)
Smoking	6 (18.1)	5 (15.1)	18 (24.0)	13 (17.3)
BMI (kg/m^2)	30.0 ± 6.3	27.8 ± 4.2	28.5 ± 4.5	27.9 ± 4.5
Systolic blood pressure (mmHg)	137 ± 15	134 ± 20	139 ± 19	137 ± 18
Diastolic blood pressure (mmHg)	75 ± 9	75 ± 8	77 ± 8	78 ± 9
History of coronary heart disease$	7 (21.3)	2 (6.1)†	11 (14.9)	9 (12.0)
Follow-up time	2.7 (2.2–3.8)	2.8 (2.3–4.0)	2.8 (2.3–4.0)	2.8 (2.1–3.7)
Laboratory parameters				
UAE (mg/24 h)	22 (17–34)	12 (6–40)*	22 (13–83)	11 (7–37)‡
eGFR (mL/min/1.73 m^2)	76 ± 18	80 ± 17	72 ± 21	69 ± 20
Total cholesterol (mmol/L)	5.0 ± 1.4	5.2 ± 1.4	5.3 ± 1.0	5.4 ± 1.1
Fasting plasma glucose (mmol/L)	7.6 ± 1.9	7.2 ± 1.2	5.3 ± 0.8	5.1 ± 0.9
Treatment				
Antihypertensive drugs	23 (69.7)	11 (33.3)	75 (100)	75 (100)
ACEi/ARB	16 (48.5)	2 (6.1)*	39 (52.0)	39 (52.0)
Oral antidiabetes medications	24 (72.7)	24 (72.7)	0 (0)	0 (0)
Lipid-lowering drugs	18 (54.6)	7 (21.2)†	27 (36.0)	20 (26.7)
Albuminuria stage				
Normoalbuminuria	24 (72.7)	24 (72.7)	50 (66.7)	50 (66.7)
Microalbuminuria	9 (27.3)	9 (27.3)	25 (33.3)	25 (33.3)
Median change in UAE (mg/24 h)	31 (22–151)	0 (−4 to 3)‡	70 (29–310)	0 (−2 to 8)‡
Median change in UAE (%)	164 (106–340)	0 (−23 to 38)	314 (143–668)	5 (−18 to 46)

Data are *n* (%), means ± SD or, for non–normally distributed variables, median (IQR) unless otherwise indicated. To convert values for serum cholesterol from millimoles per liter to milligrams per deciliter, multiply by 38.67; to convert values for fasting plasma glucose from millimoles per liter to milligrams per deciliter, multiply by 18; and to convert values for serum creatinine from micromoles per liter to milligrams per deciliter, divide by 88.4. $Self-reported coronary heart disease. †Case vs. control subjects $P < 0.01$. *Case vs. control subjects $P < 0.05$. ‡Case vs. control subjects $P < 0.001$.

consisting of UAE and eGFR (increase from 0.87 to 0.92, $P = 0.03$). GDF-15 also improved the IDI (0.148, $P = 0.001$), indicating that GDF-15 improved discrimination between case and control subjects beyond baseline UAE and eGFR.

GDF-15 in nondiabetic HT patients

The replication study was a second nested case-control study, which consisted of 75 (nondiabetic) case subjects with HT and transition to increasing stages of albuminuria who were pair matched to 75 control subjects with HT and stable albuminuria (Table 1). Characteristics of patients with HT were remarkably similar to those of patients with T2DM, as was the median follow-up time (2.8 years [IQR 2.3–3.9] in HT vs. 2.7 years [2.2–4.0] in T2DM). Case subjects with HT had a median increase in albuminuria of 70 mg/24 h (314%) compared with 0.2 mg/24 h (5%) in control subjects.

GDF-15 concentration in patients with HT was similar to the concentration in patients with T2DM (median 910 [IQR 738–1,210] vs. 992 [799–1,347]; $P = 0.63$). In case subjects with HT, GDF-15 concentration was borderline significantly higher than in control subjects (975 pg/mL [739–1,222] vs. 872 [726–1,172]; $P = 0.09$). The odds of transition to micro- or macroalbuminuria was significantly increased per SD increment in GDF-15 (odds ratio 1.96 [95% CI 1.12–3.45]). Also, in patients with HT no difference was observed in the odds for transition from normo- to microalbuminuria or micro- to macroalbuminuria with each increment in GDF-15 (P for interaction = 0.90). Addition of GDF-15 to the prediction model of transition in albuminuria, consisting of baseline UAE and eGFR, did not result in significant improvement of the *C* statistic (from 0.88 to 0.89, $P = 0.8$) but significantly improved IDI (0.059, $P = 0.04$).

Sensitivity analyses

In a multivariate model with additional adjustment for risk markers known to be associated with progression in albuminuria and with baseline differences between case and control subjects (baseline albuminuria, ACEi/ARB use, use of lipid-lowering drugs, and history of coronary heart disease), GDF-15 remained statistically significantly associated with progression in albuminuria both in T2DM (odds ratio 4.84 [95% CI 1.29–18.3], $P = 0.02$]) and in HT (1.73 [1.02–2.95], $P = 0.02$]) per SD increment in GDF-15. Because of the risk of overfitting the multivariate model, we were unable to include more risk markers for progression in our multivariate model. After combination of patients with T2DM and HT, the odds ratio for transition in albuminuria was 2.63 (95% CI 1.46–4.75), $P = 0.001$, for each SD increase in GDF-15, after adjustment for baseline albuminuria, eGFR, systolic and diastolic blood pressure, cholesterol, fasting plasma glucose, BMI, smoking, ACEi/ARB use, use of lipid-lowering drugs, and history of coronary heart disease.

CONCLUSIONS—To the best of our knowledge, this is the first study to indicate that GDF-15 precedes and predicts the development of micro- or macroalbuminuria in patients with T2DM. GDF-15 was not only independently associated with transition in albuminuria during a follow-up of ~3 years but also improved discrimination between patients who did and did not develop micro- or macroalbuminuria beyond conventional risk markers. These findings were extended and replicated in a cohort of nondiabetic HT patients.

Previous studies mainly implicated GDF-15 as a predictor for cardiovascular events and all-cause mortality in patients with previous myocardial infarction (10–12). The only previous study focusing on GDF-15 and renal outcome was performed by Lajer et al. (13) in patients

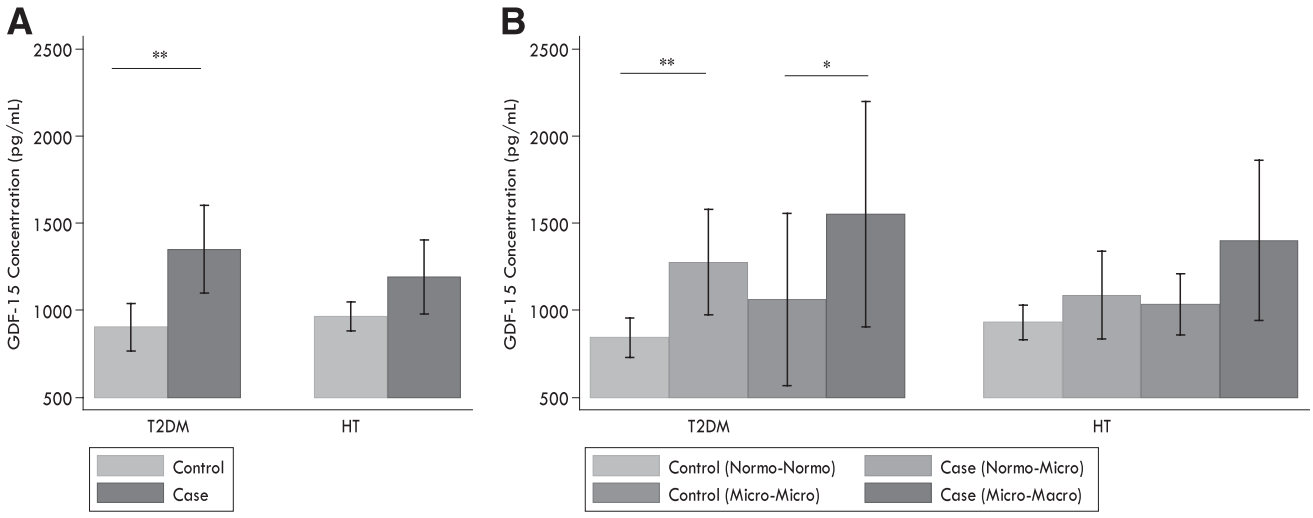

Figure 2—*GDF-15 levels in progressors and nonprogressors in albuminuria (case and control subjects) for patients with T2DM and nondiabetic patients with HT.* A: *GDF-15 levels (pg/mL) (mean ± SE) in progressors and nonprogressors in albuminuria for T2DM and HT.* B: *GDF-15 levels (pg/mL) (mean ± SE) in progressors and nonprogressors in albuminuria for T2DM and HT stratified for baseline albuminuria (normoalbuminuria [Normo] or microalbuminuria [Micro]);* *P < 0.05, **P < 0.001. *Macro, macroalbuminuria.*

with type 1 diabetes mellitus and macroalbuminuria. In this observational study, higher GDF-15 levels were associated with a faster decline of eGFR and higher risk of development of end-stage renal disease. Our study extends these latter findings to earlier stages of renal disease during which appropriate intervention is most beneficial. Future validation studies in larger cohorts are needed to confirm the value of GDF-15 in prediction of renal disease progression and to define an optimal threshold for risk of progression in the different stages of renal disease.

Despite a growing body of literature demonstrating that GDF-15 is a valuable biomarker for cardiovascular disease, relatively little is known on the pathophysiological role of GDF-15. Its expression is markedly increased in response to injury in various tissues, including heart and kidney, and is believed to be a protective factor by reducing apoptosis and influencing cellular proliferation (18–20). Higher levels of GDF-15 may thus indicate (early) tissue damage. As endothelial cells appear to be a prominent source of GDF-15, the circulating levels of GDF-15 most likely represent generalized endothelial and microvascular damage (21). The relation of GDF-15 with microvasculature may explain the link with micro- or macroalbuminuria, as increased albuminuria supposedly reflects established microvascular damage in the renal and peripheral vasculature (22). Alternatively, as GDF-15 correlates with age and decreased telomere length in the general population, GDF-15 may be a marker of (vascular) aging. In diabetes and hypertension, stress factors such as inflammation, oxidative stress, and dyslipidemia could contribute to accelerated aging, indicating that circulating levels of GDF-15 may reflect the relative age of the vasculature and its ability to resist leakage of albumin (23). Regardless, since our case and control subjects were at the same stage of albuminuria (either normo- or microalbuminuria) at the time of GDF-15 measurement, GDF-15 seems to already be increased before microalbuminuria becomes detectable. Our finding that GDF-15 also contributed in identifying subjects at risk for transition in albuminuria stage in a nondiabetic HT cohort implies that GDF-15 is not specifically related to diabetes. Future research will be necessary to delineate the exact pathophysiological role of GDF-15 in diabetes or HT and to assess its relation with albuminuria.

Traditional risk factors associated with transition in albuminuria are age, sex, dyslipidemia, insulin sensitivity, hyperglycemia, duration of diabetes, increased blood pressure, duration of hypertension, BMI, and smoking (7,24). Despite identification of these important risk factors for transition in albuminuria stage, the ability to stratify subjects at risk is still limited. The current data, if confirmed, raise the possibility of identification of subjects at risk even when traditional risk factors do not indicate risk, facilitating early identification of those with an increased chance of developing micro- or macroalbuminuria. Because early intervention

Table 2—*Odds ratios for GDF-15 and transition in albuminuria per log-unit increase and per SD increase in the level of the biomarker for patients with T2DM and nondiabetic patients with HT*

	Per doubling		Per SD increase		
	Odds ratio	95% CI	Odds ratio	95% CI	*P*
T2DM GDF-15					
Crude	7.66	1.94–30.14	3.58	1.51–8.47	0.004
Adjusted model*	5.39	1.16–25.05	2.87	1.10–7.53	0.03
HT GDF-15					
Crude	1.58	0.92–2.73	1.33	0.95–1.88	0.10
Adjusted model*	2.94	1.20–7.20	1.96	1.12–3.45	0.02

Values were calculated with conditional logistic regression analyses. The biomarkers were modeled as continuous (log-transformed) variables. *P* values are for comparison of the models with the baseline model. *Established model: prediction model based on UAE and eGFR.

is more effective than late intervention (4), preventive treatment could thus reduce the risk of renal and cardiovascular events in patients with T2DM (5,6). Our findings also applied to nondiabetic HT patients, in whom albuminuria is also strongly associated with renal and cardiovascular events (25,26).

We incorporated prediction analyses in the current study to determine whether GDF-15 could add to risk prediction in individual subjects beyond traditional risk markers. Whereas the *C* statistic is most commonly used, the *C* statistic is not very sensitive in detecting small but meaningful contributions of biomarkers in correctly classifying individuals. Hardly any improvement of the *C* statistic can be reached when the model already includes one or several important risk markers (17). Yet, we found improvement in *C* statistic in our study in T2DM. Because of the insensitivity of the *C* statistic, other measures of risk classification such as the IDI have recently been proposed (15,16). In our analyses, we also found significant improvement in IDI for GDF-15, both in patients with T2DM and in nondiabetic subjects with HT.

The availability of patient samples from the large general population cohort PREVEND was a unique feature of this study. Because this study followed the natural course of albuminuria by performing repeated measurements of albuminuria for >10 years, we were able to measure GDF-15 in samples prior to the transition in albuminuria, whereas many studies have a cross-sectional design and test biomarkers in patients with established increased albuminuria. Other strengths include the well-defined phenotype of the population, with albuminuria status based on two consecutive 24-h urine collections, the rigorous definitions for transition in albuminuria stage, and the availability of samples that had never been thawed. Because of these strict criteria and the limited number of patients with diabetes in this general population cohort, we were only able to obtain 33 valid cases.

A limitation of this study was that there was a modest difference in baseline albuminuria. This small baseline difference in albuminuria was inherent to the design of the study, as the level of albuminuria in itself is related to transition to increasing stages of albuminuria and we matched by albuminuria stage rather than by albuminuria level. Adjustment for baseline albuminuria showed that the association of GDF-15 with albuminuria transition was independent of baseline albuminuria. A second limitation is the fact that the nested case-control design of the study may overestimate the true predictive capacity of the models because of the relative high event rate in case-control studies (27). Absolute values of the *C* statistic should therefore be interpreted with caution. The improvement of the *C* statistic and the level of significance, however, are unaffected by case-control design of studies. Lastly, whereas these results are promising and could be confirmed in nondiabetic HT patients, replication of the current results in other studies, preferably large prospective cohort studies, is warranted before final conclusions can be reached.

In conclusion, we identified GDF-15 as a marker for prediction of transition in albuminuria in T2DM subjects. GDF-15, moreover, had significant additive value on top of conventional risk markers in the prediction of albuminuria transition. These findings were confirmed in nondiabetic HT subjects. If these findings prove to be replicable in other studies, GDF-15 might be a valuable marker for individual risk stratification, facilitating start or intensification of treatment in high-risk patients to prevent or delay the progression of nephropathy.

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M.E.H. developed and formulated the research questions, conducted the analysis, wrote the manuscript, contributed to discussion, and reviewed and edited the manuscript. M.M. developed and formulated the research questions, contributed to the acquisition of data, contributed to discussion, and reviewed and edited the manuscript. R.T.G. contributed to discussion and reviewed and edited the manuscript. D.d.Z. developed and formulated the research questions, contributed to the analysis and interpretation of data, contributed to discussion, and reviewed and edited the manuscript. S.J.L.B. developed and formulated the research questions, contributed to discussion, and reviewed and edited the manuscript. H.J.L.-H. developed and formulated the research questions, conducted the analysis, contributed to the analysis and interpretation of data, contributed to discussion, and reviewed and edited the manuscript. L.E.D. developed and formulated the research questions, contributed to discussion, and reviewed and edited the manuscript. M.E.H. and H.J.L.-H. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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