Circulating Fibroblast Growth Factor 21 Levels Predict Progressive Kidney Disease in Subjects With Type 2 Diabetes and Normoalbuminuria

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Background: Elevated fibroblast growth factor 21 (FGF21) levels have been suggested, from cross-sectional studies, as an indicator of subclinical diabetic nephropathy. We investigated whether serum FGF21 was predictive of the development of diabetic nephropathy.

Method: Baseline serum FGF21 levels were measured in 1136 Chinese type 2 diabetic subjects recruited from the Hong Kong West Diabetes Registry. The role of serum FGF21 in predicting decline in estimated glomerular filtration rate (eGFR) over a median follow-up of 4 years was analyzed using Cox regression analysis.

Results: At baseline, serum FGF21 levels increased progressively with eGFR category (P for trend $<.001$). Among 1071 subjects with baseline eGFR $\geq$ 30 mL/min/1.73 m^2, serum FGF21 levels were significantly higher in those with eGFR decline during follow-up (n = 171) than those without decline (n = 900) ($P < .001$). In multivariable Cox regression analysis, baseline serum FGF21 was independently associated with eGFR decline (hazard ratio, 1.21; 95% confidence interval [CI], 1.01–1.43; $P = .036$), even after adjustment for baseline eGFR. In a subgroup of 559 subjects with baseline eGFR $\geq$60 mL/min/1.73 m^2 and normoalbuminuria, serum FGF21 level remained an independent predictor of eGFR decline (hazard ratio, 1.36; 95% CI, 1.06–1.76; $P = .016$). Integrated discrimination improvement (IDI) suggested that the inclusion of baseline serum FGF21 significantly improved the prediction of eGFR decline (IDI, 1%; 95% CI, 0.1–3.0; $P = .013$) in this subgroup, but not in the initial cohort involving all subjects.

Conclusions: Elevated serum FGF21 levels may be a useful biomarker for predicting kidney disease progression, especially in the early stages of diabetic nephropathy. **(*J Clin Endocrinol Metab* 100: 1368–1375, 2015)**

Fibroblast growth factor 21 (FGF21), a circulating factor predominantly secreted from the liver, has been shown as an emerging metabolic regulator in various clinical conditions (1). Circulating FGF21 levels are elevated in patients with obesity-related disorders, including type 2 diabetes mellitus (DM) (2), metabolic syndrome (3), polycystic ovarian syndrome (4), dyslipidemia, coronary heart disease (5), and nonalcoholic fatty liver disease (6). Our previous work has also shown that high serum FGF21 levels independently predicted the development of incident type 2 DM in a cohort of community-dwelling Chinese adults (7). In addition, we have demonstrated the

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Abbreviations: AUC, area under the curve; BMI, body mass index; BP, blood pressure; CI, confidence interval; CKD, chronic kidney disease; CRP, C-reactive protein; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; FGF21, fibroblast growth factor 21; HbA1c, glycated hemoglobin; HR, hazard ratio; IDI, integrated discrimination improvement; IQR, interquartile range; NRI, net reclassification improvement; PPAR, peroxisome proliferator-activated receptor; WC, waist circumference.

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association between elevated serum FGF21 levels and carotid atherosclerosis in Chinese subjects, independent of established cardiovascular risk factors including adverse lipid profile and C-reactive protein (CRP) (8).

In the context of diabetic nephropathy, serum FGF21 levels are increased in patients with impaired renal function, and its levels are shown to increase with the progression of chronic kidney disease (CKD) (9, 10). Furthermore, FGF21 levels are also independently associated with urinary albumin excretion in Chinese patients with type 2 diabetes with macroalbuminuria or microalbuminuria (11). However, because most previous studies on FGF21 and CKD were of cross-sectional design and FGF21 is excreted mainly by the kidneys (9), the causal relationship between elevated serum FGF21 and impaired renal function remains unclear. Animal studies have demonstrated various beneficial effects on metabolic parameters after the therapeutic administration of recombinant FGF21. Furthermore, 3 months of recombinant FGF21 treatment had been shown to decrease albuminuria and improve kidney histology in diabetic mice, despite the presence of impaired FGF21 signaling in their kidneys (12). Taking all

Table 1. Baseline Characteristics of the 1071 Subjects by eGFR Decline

	eGFR Decline		
Baseline Parameters	**Yes**	**No**	***P* Value**
n	171	900	
Sex, men, %	52.6	58.3	.171
Age, y	57.2 ± 8.66	54.5 ± 9.32	**<.001**
BMI, kg/m^2	26.2 ± 4.64	25.8 ± 4.52	.242
WC, cm			.013[b]
Males	93.0 ± 11.1	90.9 ± 10.8	
Females	89.4 ± 12.7	86.6 ± 12.4	
Current smoker, %	14.0	14.0	.990
Ever smoker, %	37.4	32.4	.205
Systolic BP, mm Hg	139.1 ± 20.3	130.1 ± 19.2	**<.001**
Diastolic BP, mm Hg	77.7 ± 10.5	78.5 ± 9.57	.328
Hypertension, %	92.4	72.1	**<.001**
Antihypertensive drug, %	90.6	68.1	**<.001**
DM duration, y			**.001**
≤10 y	32.2	44.9	
11–20 y	42.1	39.9	
>20 y	25.7	15.2	
Fasting glucose, mmol/L	8.53 ± 2.65	8.34 ± 2.68	.381
HbA1c, %	8.63 ± 1.73	8.28 ± 1.56	**.008**
Serum creatinine, μmol/L[a]			**<.001[b]**
M	96.0 (78.5–127.3)	82.0 (74.0–96.0)	
F	69.0 (56.0–79.0)	62.0 (55.0–73.0)	
eGFR, mL/min/1.73 m^{2a}	72.6 (56.5–93.3)	85.1 (70.7–98.3)	**<.001**
Urinary albumin excretion, %			**<.001**
Normal	33.5	72.2	
Microalbuminuria	29.7	21.8	
Clinical albuminuria	36.8	6.0	
Cholesterol, mmol/L	4.93 ± 1.19	4.72 ± 0.95	.011
Triglyceride, mmol/L[a]	1.57 (1.19–2.10)	1.28 (0.91–1.90)	**<.001**
HDL-cholesterol, mmol/L	1.19 ± 0.38	1.20 ± 0.34	.907
LDL-cholesterol, mmol/L	2.90 ± 0.95	2.80 ± 0.83	.146
Lipid-lowering drug, %	33.3	30.6	.472
FGF21, pg/mL[a]			**<.001[b]**
M	232.5 (116.5–405.1)	160.8 (77.9–285.7)	
F	243.5 (157.2–377.1)	193.8 (108.6–327.6)	
Adiponectin, μg/mL[a]			.011[b]
M	9.10 (6.64–14.4)	8.17 (5.39–12.2)	
F	11.3 (7.70–18.3)	10.4 (7.11–15.3)	
CRP, mg/L[a]	1.84 (0.92–4.31)	1.34 (0.50–2.96)	<.001

Abbreviations: M, male; F, female; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Data are presented as mean ± SD or median (IQR). Bold *P* values indicate a statistically significant difference after Bonferroni correction for multiple testing. Conversion factors are: glucose from mmol/L to mg/dL, × 18; creatinine, from mg/dL to μmol/L, × 88.4; cholesterol/HDL/LDL-cholesterol, mmol/L to mg/dL, × 38.9; triglyceride, mmol/L to mg/dL, × 88.2.

[a] Log-transformed before analysis.

[b] Sex-adjusted *P* value.

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previous studies and evidence together, we postulate that renal FGF21 resistance, as demonstrated in the diabetic obese mice, may also be present in humans with type 2 diabetes.

In this prospective study, we investigated whether serum FGF21 level could be usefully employed as a potential biomarker in the prediction of estimated glomerular filtration rate (eGFR) decline, especially in patients at the early stages of diabetic nephropathy.

Subjects and Methods

Subjects

Subjects were recruited from the Hong Kong West Diabetes Registry, which included patients who had type 2 DM and were being followed up at the medical specialist clinics of the Hong Kong West Cluster from 2008 to 2013. All subjects were invited, with informed consent, to participate in a prospective study to identify the risk factors, including serum and genetic biomarkers, predisposing to the development of diabetic complications. Informed consent was obtained from about 85% of the subjects. Each visit was comprised of clinical assessments and laboratory investigations to determine the control of diabetes and its related cardiovascular risk factors and the presence of diabetic complications.

In this study, only subjects who attended regular follow-up visits for at least 2 years, with the latest follow-up in October 2013 or before, were included. Inclusion criteria also encompassed being Chinese and aged ≥30 years. Patients who were on dialysis or had received a kidney transplant were excluded. Therefore, a total of 1136 type 2 DM subjects were included in the study. (For details, see Supplemental Data.)

Endpoint definitions

The primary endpoint was eGFR decline, which was defined as a decline in eGFR category (≥90 [stage 1], 60–89 [stage 2], 45–59 [stage 3a], 30–44 [stage 3b], 15–29 [stage 4], and <15 [stage 5] mL/min/1.73 m^2), accompanied by a 25% or greater deterioration in eGFR from baseline (13). eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) Study formula for Chinese and expressed in mL/min/1.73 m^2: $eGFR = 175 \times [\text{serum creatinine (in } \mu\text{mol/L)} \times 0.011]^{-1.234} \times [\text{age}]^{-0.179}$ (×0.79, if female) (14).

Clinical and biochemical assessments

Subjects attended each visit after an overnight fast of at least 8 hours. At the baseline visit, demographic data, including age, sex, occupation, smoking, alcohol consumption, and physical activity were obtained. Detailed family, medical, and drug histories were ascertained using a standardized questionnaire. Anthropometric parameters, including body weight, height, body mass index (BMI), waist circumference (WC), and blood pressure (BP) were measured. Fasting blood was drawn for plasma glucose, lipids, glycated hemoglobin (HbA1c), serum creatinine, and CRP. Serum FGF21 and adiponectin levels were measured with ELISA kits (Antibody and Immunoassay Services, University of Hong Kong) as previously described (3, 8, 15). The intra- and interassay coefficients of variation of the FGF21 ELISA were 4–5 and 3.5–10.2, respectively, whereas the intra- and interassay coefficients of variation of the adiponectin ELISA were 3.27–4.02 and 4.53–5.01, respectively.

Microalbuminuria or albuminuria was defined by a urine albumin:creatinine ratio of 30 μg/mg creatinine (or 3.5 mg/mmol creatinine) or above in at least two random urine samples collected on two separate occasions within 6 months (16).

The study protocol was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. All subjects gave written informed consent before any study-related procedures.

Statistical analysis

All data were analyzed with IBM SPSS Statistics 19.0 and R-programming language version 3.1.1. (Package: pROC, survIDINRI, powerSurvEpi). Data that were not normally distributed, as determined using the Kolmogorov-Smirnov test, were natural-logarithmically transformed to obtain near normality before analysis. Values were reported as means ± SD or median (interquartile range [IQR]) as appropriate. ANOVA test with trend *P* value was used to compare the eGFR categories across FGF21 levels. χ^2 test and ANOVA were used for comparisons of categorical and continuous variables, respectively.

Multivariable Cox regression analysis was used to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) for eGFR decline. The variables included in Cox regression models were those that were statistically significant in univariate analysis, after correcting for multiple comparisons by Bonferroni correction, or were biologically relevant. Using scale Schoenfeld residuals, assumption of proportional hazard was validated for all covariates (global test) with each variable. All Cox regression models did not violate the assumption in our analyses ($P > .05$).

Table 2. Multivariable Cox Regression Analysis Showing the Association of Baseline FGF21 Level With eGFR Decline in Subjects With Baseline eGFR ≥30 mL/min/1.73 m^2

Baseline Variables	HR (95% CI)	*P* Value
Age, y	1.02 (1.00–1.04)	.130
BMI, kg/m^2	0.99 (0.95–1.03)	.643
DM duration, y	Referent	**.007**
≤10	1.55 (1.07–2.25)	**.021**
11–20	1.96 (1.27–3.04)	**.002**
>20		
HbA1c, %	0.93 (0.85–1.02)	.128
Hypertension, %	2.54 (1.44–4.68)	**.001**
eGFR, mL/min/1.73 m^2[a]	0.86 (0.53–1.40)	.541
Microalbuminuria/clinical albuminuria, %[b]	2.87 (2.05–4.02)	**<.001**
Adiponectin, μg/mL[a]	1.18 (0.89–1.55)	.249
CRP, mg/L[a]	1.32 (1.15–1.52)	**<.001**
FGF21, pg/mL[a]	1.21 (1.01–1.43)	**.036**

−2 Log likelihood of the model was 1624.949 with $P < .0001$; model included the statistically significant variables (age, DM duration, BMI, HbA1c, hypertension, eGFR, microalbuminuria/clinical albuminuria, CRP) and the biologically relevant variables (BMI and adiponectin). Bold indicates statistically significant data.

[a] Log-transformed before analysis.

[b] n = 1069.

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The predictive performance of various models was assessed by receiver operating characteristic curve, category-free net reclassification improvement (NRI), and integrated discrimination improvement (IDI). Comparison between two areas under the receiver operating characteristic curves (AUC) was examined using the DeLong method (17), and its mean difference with 95% confidence level was presented. The power calculation for Cox regression was determined using the equation proposed by Hsieh and Lavori (18). (See Supplemental Data for details.) In all statistical tests, two-sided P values $<.05$ were considered significant.

Results

Among the 1136 recruited type 2 DM subjects, at baseline, serum FGF21 levels increased progressively with categories of declining eGFR, with their median values being 163.0, 199.4, 233.7, 526.2, and 835.4 pg/mL for eGFR ≥90, 60–89, 30–59, 15–29, and <15 mL/min/1.73 m^2, respectively (P for trend <.001). Supplemental Table 1 summarizes the correlation analysis between serum FGF21 levels and other baseline parameters. Although serum FGF21 levels are known to be increased by exercise (19), data on the relationship between serum FGF21 and physical activity were not available in this study because only a crude assessment on physical activity was performed. In multiple linear regression analysis, serum FGF21 level was independently associated with sex ($P <$ .001), BMI ($P < .001$), eGFR ($P < .001$), microalbuminuria or clinical albuminuria ($P = .003$), dyslipidemia ($P = .003$), adiponectin ($P < .001$), and CRP ($P = .041$) (Supplemental Table 2).

After exclusion of 39 subjects with less than 2 years of follow-up and 26 subjects with eGFR <30 mL/min/1.73 m^2, of the remaining 1071 subjects with baseline eGFR ≥30 mL/min/1.73 m^2, 171 (16%) subjects had eGFR decline over a median follow-up of 4 years. Subjects with eGFR decline were significantly older, they had longer duration of DM, with more of them being hypertensive and on antihypertensive medications, and they had higher systolic BP, lower baseline eGFR, and higher serum triglyceride, CRP, and adiponectin levels than those without eGFR decline (n = 900) (Table 1). Among those with eGFR decline, only 33.5% of subjects had normal urinary albumin excretion, as compared with 72.2% in those without eGFR decline. Serum FGF21 levels were significantly higher in those with eGFR decline during follow-up than those without decline ($P < .001$). In addition, baseline FGF21 levels negatively correlated with change in eGFR (Crude R = −0.077; $P = .012$).

In multivariable Cox regression analysis, baseline serum FGF21 level was independently associated with eGFR decline (HR, 1.21; 95% CI, 1.01–1.43; $P = .036$), even after adjustment for potential confounders including baseline eGFR (Table 2). Other significant independent predictors of eGFR decline included duration of diabetes ≥20 years (HR, 1.96; 95% CI, 1.27–3.04; $P = .002$), hypertension (HR, 2.54; 95% CI, 1.44–4.68; $P = .001$), microalbuminuria or albuminuria (HR, 2.87; 95% CI, 2.05–4.02; $P < .001$), and CRP (HR, 1.32; 95% CI, 1.15–1.52; $P < .001$). There were 81 subjects who were on peroxisome proliferator-activated receptor (PPAR) α-agonists at baseline and who remained on the same dosage during the follow-up period. No subject was on PPARγ-agonists. After exclusion of the 81 subjects on PPARα-agonist, which might impact on the FGF21 levels (20), baseline FGF21 levels still remained a significant predictor of eGFR decline on multivariate Cox regression analysis after adjusting for age, DM duration, presence of hypertension, HbA1c, eGFR, microalbuminuria or clinical albuminuria, CRP, BMI, and adiponectin (HR, 1.28; 95% CI, 1.05–1.56; $P = .015$). However, in a clinical model that was comprised of age, duration of diabetes, hypertension, HbA1c, eGFR, microalbuminuria or clinical albuminuria, and BMI, the addition of baseline serum FGF21 did not produce a significant improvement in the prediction of eGFR decline. The mean difference in the two AUC values was 1% (95% CI, −0.2 to 2.1), with a category-free NRI of 8.3% (95% CI, −10.8 to 21.0; $P = .312$) and an IDI of 0.3% (95% CI, −0.4 to 1.7; $P = .359$) (Table 3).

In a subgroup of 559 subjects with normoalbuminuria and baseline eGFR ≥60 mL/min/1.73 m^2, 45 (8%) subjects had eGFR decline on follow-up. Baseline parameters including age, eGFR, duration of diabetes, and HbA1c were similar between groups with or without eGFR decline. However, subjects with eGFR decline had significantly higher prevalence of hypertension ($P < .001$), CRP ($P = .001$), and sex-adjusted FGF21 levels ($P = .009$) at baseline than those without eGFR decline (Table 4). In multivariable Cox regression analysis, baseline serum FGF21 was an independent predictor of eGFR decline in this subgroup of patients (HR, 1.36; 95% CI, 1.06–1.76; $P = .016$), together with hypertension (HR, 8.18; 95% CI, 2.52–26.5; $P < .001$), and CRP (HR, 1.40; 95% CI, 1.04–1.88; $P = .028$) (Table 5). Notably, contrary to the original cohort, in this subgroup of subjects, the addition of baseline serum FGF21 to hypertension provided a significant improvement in the prediction of eGFR decline, as reflected by a mean difference in the two AUC values of 7.9% (95% CI, 3.1–12.7) and an IDI of 1% (95% CI, 0.1–3.0; $P = .013$). The addition of CRP to hypertension in the clinical model also yielded similar improvement in outcome prediction (Table 6). Furthermore, the addition of CRP to the model including hypertension and FGF21

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Table 3. Discrimination and Reclassification of eGFR Decline With Prediction Multivariable Cox Regression Model Among All Subjects With Baseline eGFR ≥30 mL/min/1.73 m^2

Additional Variable to the Reduced Model (n = 1069)	Mean Difference in 2 AUC (95% CI)	Category-Free NRI (95% CI)	IDI (95% CI)
+FGF21	1.0% (−0.2 to 2.1) Delong P = .092	8.3% (−10.8 to 21.0) P = .312	0.3 (−0.4 to 1.7) P = .359

Reduced model: age, DM duration, HbA1c, hypertension, eGFR, microalbuminuria/clinical albuminuria, and BMI.

provided significant yet comparable increments in the prediction of eGFR decline, compared to the addition of FGF21 to the model including hypertension and CRP, as reflected by an IDI of 1.6% (95% CI, 0.1–6.5; P = .027; after addition of CRP to the clinical model of hypertension and FGF21) and 1% (95% CI, 0–3.8; P = .04; after addition of FGF21 to the clinical model of hypertension and CRP).

Table 4. Baseline Characteristics of the Subgroup of Diabetic Subjects With Baseline eGFR ≥60 mL/min/1.73 m^2 and Normoalbuminuria by eGFR Decline

	eGFR Decline		
Baseline Parameters	**Yes**	**No**	***P* Value**
n	45	514	
Sex, men, %	37.8	53.3	.046
Age, y	56.0 ± 7.18	53.7 ± 8.94	.082
BMI, kg/m^2	26.1 ± 5.10	24.9 ± 4.11	.072
WC, cm			.057[b]
M	89.9 ± 10.5	88.2 ± 10.1	
F	88.8 ± 14.6	84.4 ± 11.4	
Current smoker, %	13.3	13.6	.957
Ever smoker, %	24.4	27.8	.627
Systolic BP, mm Hg	135.3 ± 16.5	126.9 ± 17.6	.002
Diastolic BP, mm Hg	77.2 ± 8.25	77.2 ± 8.78	.975
Hypertension, %	93.3	61.5	**<.001**
Antihypertensive drug, %	91.1	57.2	**<.001**
DM duration, %			.193
≤10 y	46.7	35.6	
11–20 y	39.5	53.3	
>20 y	13.8	11.1	
Fasting glucose, mmol/L	8.24 ± 2.27	8.18 ± 2.48	.880
HbA1c, %	8.42 ± 1.48	8.13 ± 1.40	.185
Cholesterol, mmol/L	4.86 ± 1.41	4.75 ± 0.92	.469
Triglyceride, mmol/L[a]	1.51 (1.05–1.91)	1.20 (0.86–1.85)	.134
HDL-cholesterol, mmol/L	1.25 ± 0.32	1.23 ± 0.35	.744
LDL-cholesterol, mmol/L	2.86 ± 1.08	2.83 ± 0.82	.828
Lipid-lowering drug, %	24.4	25.3	.900
Serum creatinine, μmol/L[a]			.914[b]
M	77.0 (64.5–92.0)	80.0 (73.0–88.0)	
F	61.5 (55.3–71.5)	61.0 (55.0–67.0)	
eGFR, mL/min/1.73 m^2[a]	87.9 (74.6–99.4)	88.3 (78.1–100.1)	.952
FGF21, pg/mL[a]			**.009**[b]
M	184.4 (68.3–352.8)	141.4 (65.7–237.0)	
F	272.2 (194.1–424.8)	180.8 (107.7–313.7)	
Adiponectin, μg/mL[a]			.147[b]
M	6.78 (5.24–9.79)	8.46 (5.42–11.8)	
F	9.39 (6.27–15.1)	10.1 (7.24–14.6)	
CRP, mg/L	2.02 (0.99–3.77)	1.08 (0.45–2.48)	**.001**

Abbreviations: M, male; F, female; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Data are presented as mean ± SD or median (IQR). Bold P values indicate a statistically significant difference after Bonferroni correction for multiple testing. Conversion factors are: glucose from mmol/L to mg/dL, × 18; creatinine, from mg/dL to μmol/L, × 88.4; cholesterol/HDL/LDL-cholesterol, mmol/L to mg/dL, × 38.9; triglyceride, mmol/L to mg/dL, × 88.2.

[a] Log-transformed before analysis.

[b] Sex-adjusted P value.

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Table 5. Multivariable Cox Regression Analysis Showing the Association of Baseline Serum FGF21 Levels With eGFR Decline in 559 Subjects With Baseline eGFR ≥60 mL/min/1.73 m^2 and Normoalbuminuria

Baseline Variables	HR (95% CI)	*P* Value
Hypertension, %	8.18 (2.52–26.5)	**<.001**
CRP, mg/L[a]	1.40 (1.04–1.88)	**.028**
FGF21, pg/mL[a]	1.36 (1.06–1.76)	**.016**

−2 Log likelihood of the model was 434.224 with $P < .0001$. All statistically significant differences after Bonferroni corrections for multiple testing in the univariate analyses and biologically relevant variables were included. Bold indicates statistically significant data.

[a] Log-transformed before analysis.

Discussion

Previous cross-sectional studies have demonstrated that circulating FGF21 levels are associated with renal function, both in patients with diabetic nephropathy (10, 11) and in community-dwelling adults (21). Furthermore, its levels are also independently associated with urinary albumin excretion in type 2 diabetic patients, including those with microalbuminuria or subclinical diabetic nephropathy (11). In this prospective study, we demonstrated for the first time an independent association between elevated serum FGF21 levels and eGFR decline over a median follow-up of 4 years.

We found that serum FGF21 level was an independent predictor of eGFR decline in this prospective cohort, together with age, hypertension, duration of diabetes, microalbuminuria or clinical albuminuria, and CRP. Notably, this remained significant even after adjustment for the baseline eGFR levels of these subjects. This suggested that elevated FGF21 levels might reflect on processes that are causally related to impaired renal function in subjects with diabetic nephropathy, other than being just a consequence of reduced renal clearance, as suggested by its rise after unilateral nephrectomy (9). In agreement with previous cross-sectional studies, we also found that circulating FGF21 levels increased progressively across eGFR categories, suggesting that serum FGF21 level might remain as a biomarker to predict CKD progression even as renal function deteriorates.

Elevated circulating FGF21 levels could represent a compensatory response to FGF21 resistance or to the underlying metabolic disturbances in type 2 DM (1, 2). Animal studies had also shown that an increase in FGF21 expression might be part of a protective response to tissue stress. Johnson et al (22) demonstrated that pancreatic FGF21 expression was induced in cerulein-induced acute pancreatitis, which in turn protects pancreas from further damage. Schaap et al (23) showed that in mice with nonalcoholic fatty liver disease, endoplasmic reticulum stress could induce the expression of FGF21. We also demonstrated that in mice with acetaminophen-induced hepatotoxicity, there was a significant marked elevation in serum FGF21 levels that preceded the elevations of serum transaminases, the established liver injury markers, and that FGF21 knockout mice had greater liver damage and mortality compared to wild-type mice (24). A recent data mining analysis revealed that elevated FGF21 gene expression represented one of the early signatures of in vitro and in vivo drug toxicity in humans and rats. Early expression changes of four genes, which included FGF21, predicted drug-induced kidney and liver injuries with high accuracy (25). In the context of diabetic nephropathy, FGF21 expression in the mesangial cells of the kidneys in db/db mice was significantly up-regulated to 20-fold as compared to control db/m mice, although it remains uncertain how much of this elevation is accounted for by renal production. In addition, although animal studies had shown that circulating serum FGF21 levels were elevated, FGF21 signaling in the kidney was markedly reduced in db/db mice, as reflected by the decreased ERK phosphorylation in response to FGF21 administration, suggesting the presence of FGF21 resistance. Although this elevation of serum FGF21 levels observed in patients with early stages of diabetic nephropathy may appear paradoxical, this is in fact reminiscent of the situation of adiponectin resistance in diabetic nephropathy, or the presence of insulin resistance and hyperinsulinemia in patients with type 2 diabetes. In type 2 diabetes, despite the presence of insulin resistance, insulin remains an effective treatment. A similar scenario is likely with regard to FGF21 resistance and early diabetic nephropathy. On the other hand, elevated serum FGF21

Table 6. Discrimination and Reclassification of eGFR Decline With Prediction Multivariable Cox Regression Model Among the 559 Subjects With Baseline eGFR ≥60 mL/min/1.73 m^2 and Normoalbuminuria

Reduced Model	Additional Variable	Mean Difference in 2 AUC (95% CI)	Category-Free NRI (95% CI)	IDI (95% CI)
Hypertension	+FGF21	7.9% (3.1 to 12.7)	21.6% (−6.2 to 41.5) *P* = .100	1.0% (0.1 to 3.0) *P* = **.013**
	+CRP	8.3% (3.8 to 12.9)	27.0% (2.1 to 46.1) *P* = .020	1.0% (0.2 to 3.3) *P* = .013

Reduced model includes Hypertension variable. Bold indicates statistically significant data.

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levels might also reflect a compensatory response toward the underlying metabolic dysregulation such as insulin resistance, a known risk factor for progressive renal disease (1). Therefore, despite the high circulating serum FGF21 levels, administration of recombinant FGF21 treatment may still bring about therapeutic benefits. In fact, a renoprotective effect of exogenous FGF21 had already been clearly demonstrated in db/db mice, leading to attenuated renal injury via its beneficial effects on metabolic parameters and its antifibrotic action (12). This protective effect of FGF21 in diabetic nephropathy was confirmed in another mouse study, with the administration of FGF21 resulting in decreased renal apoptosis and suppressed diabetes-induced renal inflammation, oxidative stress, and fibrosis (26).

Diabetic nephropathy is one of the devastating complications of type 2 DM. Patients with diabetic nephropathy are at risk of progression to end-stage renal failure requiring renal replacement therapy. In addition, they are more liable to cardiovascular morbidity and mortality. The incidence of end-stage renal failure secondary to diabetic nephropathy has been increasing, partly secondary to an increase in incident diabetes worldwide. To tackle this global health problem, one potential strategy is to identify, as early as possible, those diabetic subjects at high risk of progressive nephropathy, so that intensive multifactorial risk management can be implemented in a timely fashion to prevent disease progression. Microalbuminuria has been a traditional early marker of diabetic nephropathy, but its sensitivity and specificity in predicting CKD progression have recently been questioned (27). Our study demonstrated that, even in subjects with relatively well-preserved kidney function, with an eGFR $\geq$60 mL/min/1.73 m^2 and normoalbuminuria, serum FGF21 elevation remained a reliable biomarker for eGFR decline. In this subgroup of patients, other than FGF21, only CRP and the presence of hypertension were independent predictors of eGFR decline in multivariate Cox regression analysis. We further demonstrated that the additional measurement of FGF21 could improve significantly the prediction of eGFR decline over hypertension alone. Furthermore, the improvement in eGFR decline prediction in these subjects was comparable after the addition of either CRP or FGF21. These findings suggested the potential use of serum FGF21 as a novel, alternative biomarker for the early identification of type 2 diabetic subjects who are at increased risk of progression of their kidney disease. In addition, despite the presence of renal FGF21 resistance, FGF21 may also be a potential therapeutic target to prevent eGFR decline in those with early diabetic nephropathy. Indeed, a recent study has demonstrated the beneficial effects of an FGF21 analog on hyperlipidemia, body weight, and hyperinsulinemia in type 2 diabetic patients, despite the presence of high circulating FGF21 levels suggesting FGF21 resistance (28).

Our study is limited first by the relatively short duration of follow-up relative to the time required for the progression of diabetic nephropathy. In fact, the median follow-up might be too short for proper evaluation of kidney disease progression in the whole cohort because more severe disease would progress faster. Secondly, only a single renal endpoint of CKD progression was analyzed. Other significant clinical endpoints in diabetic nephropathy including the change in levels of albuminuria or development of end-stage renal disease, requiring renal replacement therapy or renal transplantation, were not included for analysis. Furthermore, the vast majority of the subjects with CKD in our cohort did not have a renal biopsy performed to ascertain the cause of nephropathy.

Nonetheless, our study has provided the first report demonstrating serum baseline FGF21 level as an independent biomarker for predicting eGFR decline in type 2 diabetic patients at early stages of diabetic nephropathy. Further studies involving large patient cohorts with longer durations of follow-up are warranted to validate our findings.

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References

1. **Woo YC, Xu A, Wang Y, Lam KS.** Fibroblast growth factor 21 as an emerging metabolic regulator: clinical perspectives. *Clin Endocrinol (Oxf)*. 2013;78:489–496.

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2. **Chavez AO, Molina-Carrion M, Abdul-Ghani MA, Folli F, Defronzo RA, Tripathy D.** Circulating fibroblast growth factor-21 is elevated in impaired glucose tolerance and type 2 diabetes and correlates with muscle and hepatic insulin resistance. *Diabetes Care*. 2009;32:1542–1546.
3. **Zhang X, Yeung DC, Karpisek M, et al.** Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. *Diabetes*. 2008;57:1246–1253.
4. **Gorar S, Culha C, Uc ZA, et al.** Serum fibroblast growth factor 21 levels in polycystic ovary syndrome. *Gynecol Endocrinol*. 2010;26:819–826.
5. **Lin Z, Wu Z, Yin X, et al.** Serum levels of FGF-21 are increased in coronary heart disease patients and are independently associated with adverse lipid profile. *PLoS One*. 2010;5:e15534.
6. **Li H, Fang Q, Gao F, et al.** Fibroblast growth factor 21 levels are increased in nonalcoholic fatty liver disease patients and are correlated with hepatic triglyceride. *J Hepatol*. 2010;53:934–940.
7. **Chen C, Cheung BM, Tso AW, et al.** High plasma level of fibroblast growth factor 21 is an independent predictor of type 2 diabetes: a 5.4-year population-based prospective study in Chinese subjects. *Diabetes Care*. 2011;34:2113–2115.
8. **Chow WS, Xu A, Woo YC, et al.** Serum fibroblast growth factor-21 levels are associated with carotid atherosclerosis independent of established cardiovascular risk factors. *Arterioscler Thromb Vasc Biol*. 2013;33:2454–2459.
9. **Hindricks J, Ebert T, Bachmann A, et al.** Serum levels of fibroblast growth factor-21 are increased in chronic and acute renal dysfunction. *Clin Endocrinol (Oxf)*. 2014;80:918–924.
10. **Lin Z, Zhou Z, Liu Y, et al.** Circulating FGF21 levels are progressively increased from the early to end stages of chronic kidney diseases and are associated with renal function in Chinese. *PLoS One*. 2011;6:e18398.
11. **Jian WX, Peng WH, Jin J, et al.** Association between serum fibroblast growth factor 21 and diabetic nephropathy. *Metabolism*. 2012;61:853–859.
12. **Kim HW, Lee JE, Cha JJ, et al.** Fibroblast growth factor 21 improves insulin resistance and ameliorates renal injury in db/db mice. *Endocrinology*. 2013;154:3366–3376.
13. Chapter 2: Definition, identification, and prediction of CKD progression. *Kidney Int Suppl (2011)*. 2013;3:63–72.
14. **Ma YC, Zuo L, Chen JH, Luo Q, Yu XQ, Li Y, et al.** Modified glomerular filtration rate estimating equation for Chinese patients with chronic kidney disease. *J Am Soc Nephrol*. 2006;17:2937–2944.
15. **Wang Y, Lam KS, Xu JY, et al.** Adiponectin inhibits cell proliferation by interacting with several growth factors in an oligomerization-dependent manner. *J Biol Chem*. 2005;280:18341–18347.
16. **American Diabetes Association.** Standards of medical care in diabetes–2014. *Diabetes Care*. 2014;37(suppl 1):S14–S80.
17. **DeLong ER, DeLong DM, Clarke-Pearson DL.** Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*. 1988;44:837–845.
18. **Hsieh FY, Lavori PW.** Sample-size calculations for the Cox proportional hazards regression model with nonbinary covariates. *Control Clin Trials*. 2000;21:552–560.
19. **Cuevas-Ramos D, Almeda-Valdés P,, Meza-Arana CE, et al.** Exercise increases serum fibroblast growth factor 21 (FGF21) levels. *PLoS One*. 2012;7:e38022.
20. **Gälman C, Lundåsen T, Kharitonenkov A, et al.** The circulating metabolic regulator FGF21 is induced by prolonged fasting and PPARα activation in man. *Cell Metab*. 2008;8:169–174.
21. **Crasto C, Semba RD, Sun K, Ferrucci L.** Serum fibroblast growth factor 21 is associated with renal function and chronic kidney disease in community-dwelling adults. *J Am Geriatr Soc*. 2012;60:792–793.
22. **Johnson CL, Weston JY, Chadi SA, et al.** Fibroblast growth factor 21 reduces the severity of cerulein-induced pancreatitis in mice. *Gastroenterology*. 2009;137:1795–1804.
23. **Schaap FG, Kremer AE, Lamers WH, Jansen PL, Gaemers IC.** Fibroblast growth factor 21 is induced by endoplasmic reticulum stress. *Biochimie*. 2013;95:692–699.
24. **Ye D, Wang Y, Li H, et al.** Fibroblast growth factor 21 protects against acetaminophen-induced hepatotoxicity by potentiating peroxisome proliferator-activated receptor coactivator protein-1α-mediated antioxidant capacity in mice. *Hepatology*. 2014;60:977–989.
25. **Zhang JD, Berntenis N, Roth A, Ebeling M.** Data mining reveals a network of early-response genes as a consensus signature of drug-induced in vitro and in vivo toxicity. *Pharmacogenomics J*. 2014;14:208–216.
26. **Zhang C, Shao M, Yang H, et al.** Attenuation of hyperlipidemia- and diabetes-induced early-stage apoptosis and late-stage renal dysfunction via administration of fibroblast growth factor-21 is associated with suppression of renal inflammation. *PLoS One*. 2013;8:e82275.
27. **Macisaac RJ, Ekinci EI, Jerums G.** Markers of and risk factors for the development and progression of diabetic kidney disease. *Am J Kidney Dis*. 2014;63:S39–S62.
28. **Gaich G, Chien JY, Fu H, et al.** The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. *Cell Metab*. 2013;18:333–340.

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