

## Fibroblast Growth Factor 23 (FGF23) Predicts Progression of Chronic Kidney Disease: The Mild to Moderate Kidney Disease (MMKD) Study

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### ABSTRACT

It has not been firmly established whether disturbed calcium-phosphate metabolism affects progression of chronic kidney disease (CKD) in humans. In this cohort study of 227 nondiabetic patients with CKD, we assessed fibroblast growth factor 23 (FGF23) plasma concentrations in addition to other variables involved in calcium-phosphate metabolism, and we followed 177 of the patients prospectively for a median of 53 months to assess progression of renal disease. In the baseline cohort, we found a significant inverse correlation between glomerular filtration rate and both c-terminal and intact FGF23 levels (both  $P < 0.001$ ). The 65 patients who experienced a doubling of serum creatinine and/or terminal renal failure were significantly older, had a significantly lower glomerular filtration rate at baseline, and significantly higher levels of intact parathormone, c-terminal and intact FGF23, and serum phosphate (all  $P < 0.001$ ). Cox regression analysis revealed that both c-terminal and intact FGF23 independently predict progression of CKD after adjustment for age, gender, GFR, proteinuria, and serum levels of calcium, phosphate, and parathyroid hormone. The mean follow-up time to a progression end point was 46.9 (95% CI 40.2 to 53.6) months versus 72.5 (95% CI 67.7 to 77.3) months for patients with c-terminal FGF23 levels above or below the optimal cut-off level of 104 rU/mL (derived by receiver operator curve analysis), respectively. In conclusion, FGF23 is a novel independent predictor of progression of renal disease in patients with nondiabetic CKD. Its pathophysiological significance remains to be elucidated.

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Disturbed calcium-phosphate metabolism affects cardiovascular morbidity and mortality in patients with chronic kidney disease (CKD), particularly in patients with ESRD.<sup>1,2</sup> So far, it has not been firmly established whether it is also related to CKD progression. Among factors that are related to calcium-phosphate metabolism in patients with CKD, potential culprits for progression are hyperphosphatemia, hyperparathyroidism, lack of active vitamin D, and possibly excess of the recently discovered phosphaturic hormone fibroblast growth factor 23 (FGF23).<sup>3,4</sup> Early experimental work sug-

gested a parathyroid hormone (PTH)-independent beneficial role of phosphate restriction on progres-

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sion in rats,<sup>5</sup> but it has to be pointed out that these animals have physiologic hyperphosphatemia. Furthermore, there is also little direct experimental or clinical evidence for a role of PTH in accelerating progression,<sup>3</sup> although results from recent experimental studies documented that progression is significantly attenuated by administration of calcimimetics or by parathyroidectomy.<sup>6</sup> However, a confounding effect of lower BP values in these experimental settings cannot be excluded. The most solid evidence from experimental studies for an effect on progression exists for active vitamin D (1,25-OH<sub>2</sub>D<sub>3</sub>). Although in the past it was assumed that vitamin D therapy is “nephrotoxic,” probably as a result of vitamin D–induced hypercalcemia in patients with CKD,<sup>7</sup> recent experimental evidence clearly revealed that 1,25-OH<sub>2</sub>D<sub>3</sub> and its analogues attenuate progression in various CKD models.<sup>3,8,9</sup> Finally, the role of FGF23 in CKD progression is unknown.

We assessed various parameters of calcium-phosphate metabolism including c-terminal and biologically active intact FGF23 plasma concentrations in 227 patients who did not have diabetes and had primary CKD, 177 of whom were prospectively followed for a median of 53 mo. We examined the working hypothesis that FGF23 is a predictor of CKD progression.

## RESULTS

### Stages of CKD and Calcium-Phosphate Metabolism

Baseline clinical characteristics and laboratory data of 227 patients with CKD are reported in Table 1. To elucidate the relationship between renal function and parameters of calcium-phosphate metabolism, we stratified renal patients into four groups according to National Kidney Foundation (NKF) criteria for CKD: GFR  $\geq$ 90 ml/min per 1.73 m<sup>2</sup>, GFR 60 to 89 ml/min per 1.73 m<sup>2</sup>, GFR 30 to 59 ml/min per 1.73 m<sup>2</sup>, and GFR <30 ml/min per 1.73 m<sup>2</sup> (Table 1). We found a continuous and significant increase of Ca  $\times$  P, PTH, and both c-terminal and intact FGF23 concentration across the various NKF stages of renal dysfunction. In addition, serum phosphate was significantly higher in patients with more advanced renal failure. We found significant correlations between c-terminal FGF23 as well as intact FGF23 with GFR, phosphate, and PTH (Figure 1). When we analyzed the data of patients with only CKD stages 2 through 5, we found similar results as for the entire population (data not shown).

### Calcium-Phosphate Metabolism and Progression of CKD

Clinical characteristics and laboratory data of patients with follow-up are reported in Table 2. The median follow-up after completion of the baseline investigation was 53 mo (range 3 to 84 mo), and during this follow-up, 65 patients had progressed to a renal end point (33 patients had doubled their serum creatinine, and 29 had reached terminal renal failure necessitating renal replacement therapy; the two groups differed with respect to baseline serum creatinine, GFR, c-terminal FGF23, Ca  $\times$  P, and the use of phosphate binders). Patients who had

reached a progression end point were significantly older and had higher protein excretion rates and lower GFR. In addition, they had significantly higher phosphate, PTH, and FGF23 levels and Ca  $\times$  P. There were no differences for surrogate parameters of nutritional (body mass index, albumin) and inflammatory status (high-sensitivity C-reactive protein [hsCRP]).

Age- and gender-adjusted Cox regression analysis revealed that GFR, c-terminal and intact FGF23, phosphate, Ca  $\times$  P, and PTH all showed a strong association with progression-free survival (all  $P < 0.001$ ; Table 3, model 1), and proteinuria showed a weaker association ( $P = 0.03$ ). In a multivariable analysis adjusting for all variables in addition to age and gender, only baseline GFR ( $P < 0.001$ ), c-terminal FGF23 ( $P = 0.002$ ), and intact FGF23 ( $P = 0.005$ ) remained significant predictors for progression, and serum calcium, phosphate, and PTH concentrations were not independently associated with disease progression (Table 3, model 2). When we used only parameters of the calcium-phosphate metabolism without FGF23 levels in a third model, we observed PTH and Ca  $\times$  P besides baseline GFR to predict CKD progression. Phosphate concentrations were of borderline significance ( $P = 0.063$ ), and calcium was not associated with CKD progression (Table 3, model 3). To test the goodness of fit of the models to the observed data, we also analyzed models with c-terminal and intact FGF23 concentrations that were transformed using the log base 2 logarithm (log<sub>2</sub> c-terminal FGF23 or log<sub>2</sub> intact FGF23). We found that both log-transformed parameters remained significant, and results were comparable to models interpreting hazard ratios for an increment of 10 units in FGF23 concentration (data not shown).

To evaluate the operating characteristics of FGF23 as a prognostic tool for the progression of kidney disease, we performed a receiver operating characteristic (ROC) analysis for c-terminal and intact FGF23 in comparison with GFR (Figure 2). GFR obtained the highest area under the curve (AUC; AUC = 0.84; 95% confidence interval [CI] 0.78 to 0.90;  $P < 0.001$ ), followed by c-terminal FGF23 (AUC = 0.81; 95% CI 0.74 to 0.88;  $P < 0.001$ ) and intact FGF23 (AUC = 0.72; 95% CI 0.64 to 0.80;  $P < 0.001$ ). We further constructed Kaplan-Meier curves of progression-free survival comparing patients with c-terminal FGF23 values above and below the optimal cutoff level of 104 rU/ml and for intact FGF23 values above and below the median of 35 pg/ml (a value very close to the optimal cutoff derived by ROC analysis). Patients who had c-terminal and intact FGF23 levels above these threshold values had a worse prognosis and statistically significant shorter progression times compared with patients with values below the threshold (Figure 3): Mean time to progression 46.9 mo (95% CI 40.2 to 53.6) compared with 72.5 mo (95% CI 67.7 to 77.3;  $P < 0.0001$ ), respectively, for c-terminal FGF23 and 54.6 mo (95% CI 48.2 to 61.0) compared with 69.8 mo (95% CI 64.8 to 74.8;  $P = 0.0004$ ), respectively, for intact FGF23. We observed similar separation of survival curves when we used the median of c-terminal FGF23 (85 rU/ml) instead of the ROC-derived cutoff of 104 rU/ml (data not shown).

**Table 1.** Baseline clinical and laboratory data of 227 patients stratified according to GFR in NKF stages<sup>a</sup>

Parameter	GFR (ml/min per 1.73 m <sup>2</sup> )				P <sup>b</sup>
	≥90 (n = 72)	60 to 89 (n = 49)	30 to 59 (n = 63)	<30 (n = 43)	
Gender (male/female; n [%])	50/22 (69/31)	34/15 (69/31)	44/19 (70/30)	26/17 (61/40)	0.72
Age (yr)	39.9 ± 13.2	46.1 ± 11.6	45.9 ± 11.5	54.4 ± 8.5	<0.001
BMI (kg/m <sup>2</sup> )	24.0 ± 3.3	25.6 ± 3.8	25.4 ± 3.3	26.1 ± 4.8	0.02
Current smokers (n [%])	18 (25)	11 (22)	11 (18)	9 (21)	0.97
Systolic BP (mmHg)	134 ± 21	140 ± 24	139 ± 19	137 ± 19	0.21
Diastolic BP (mmHg)	84 ± 13	88 ± 15	88 ± 14	88 ± 13	0.20
Serum creatinine (mg/dl)	1.14 ± 0.22 (0.95, 1.11, 1.30)	1.54 ± 0.45 (1.25, 1.43, 1.70)	2.31 ± 0.79 (1.70, 2.18, 2.80)	3.63 ± 1.27 (2.73, 3.50, 4.61)	<0.001
GFR (ml/min per 1.73 m <sup>2</sup> )	120 ± 28 (97, 110, 132)	74 ± 9 (65, 71, 81)	44 ± 7 (38, 44, 50)	19 ± 7 (12, 18, 26)	<0.001
Proteinuria (g/24 h per 1.73 m <sup>2</sup> )	0.60 ± 0.66 (0.13, 0.36, 0.82)	1.10 ± 1.10 (0.16, 0.57, 1.93)	1.08 ± 0.94 (0.27, 0.81, 1.83)	1.03 ± 0.81 (0.36, 0.89, 1.52)	0.004
Serum albumin (g/dl)	4.70 ± 0.38	4.46 ± 0.50	4.55 ± 0.38	4.53 ± 0.34	0.01
hsCRP (mg/L)	0.21 ± 0.27	0.32 ± 0.33	0.23 ± 0.21	0.35 ± 0.38	0.01
FGF23 c-terminal (rU/ml)	57 ± 43 (36, 46, 63)	81 ± 52 (45, 69, 99)	187 ± 194 (67, 108, 230)	456 ± 475 (127, 285, 584)	<0.001
FGF23 intact (pg/ml)	29 ± 28 (27, 56, 83)	40 ± 37 (25, 43, 57)	43 ± 26 (19, 30, 44)	77 ± 83 (12, 23, 36)	<0.001
Calcium (mmol/L)	2.37 ± 0.11 (2.30, 2.36, 2.43)	2.40 ± 0.30 (2.30, 2.37, 2.43)	2.34 ± 0.15 (2.27, 2.37, 2.44)	2.34 ± 0.20 (2.23, 2.32, 2.46)	0.63
Phosphate (mmol/L)	1.02 ± 0.42 (0.85, 0.97, 1.10)	1.01 ± 0.29 (0.86, 0.97, 1.18)	1.08 ± 0.23 (0.92, 1.03, 1.18)	1.32 ± 0.24 (1.19, 1.29, 1.55)	<0.001
Ca × P (mg <sup>2</sup> /dl <sup>2</sup> )	2.40 ± 0.92 (2.02, 2.26, 2.58)	2.42 ± 0.69 (1.89, 2.39, 2.81)	2.52 ± 0.51 (2.16, 2.47, 2.83)	3.10 ± 0.63 (2.74, 2.98, 3.52)	<0.001
Parathormone (pmol/L)	3.8 ± 1.5 (2.7, 3.5, 4.9)	6.8 ± 4.3 (4.6, 5.8, 7.6)	12.0 ± 9.5 (5.3, 9.5, 17.0)	27.3 ± 21.6 (13.0, 21.0, 38.0)	<0.001
Use of vitamin D (n [%])	0 (0)	4 (8)	13 (21)	13 (30)	<0.001
Use of phosphate binder (n [%])	0 (0)	2 (4)	3 (5)	7 (16)	0.002

<sup>a</sup>Data are means ± SD and 25th, 50th (median), and 75th percentiles for skewed variables where appropriate. BMI, body mass index; FGF23, fibroblast growth factor 23; hsCRP, high-sensitivity C-reactive protein; NKF, National Kidney Foundation.

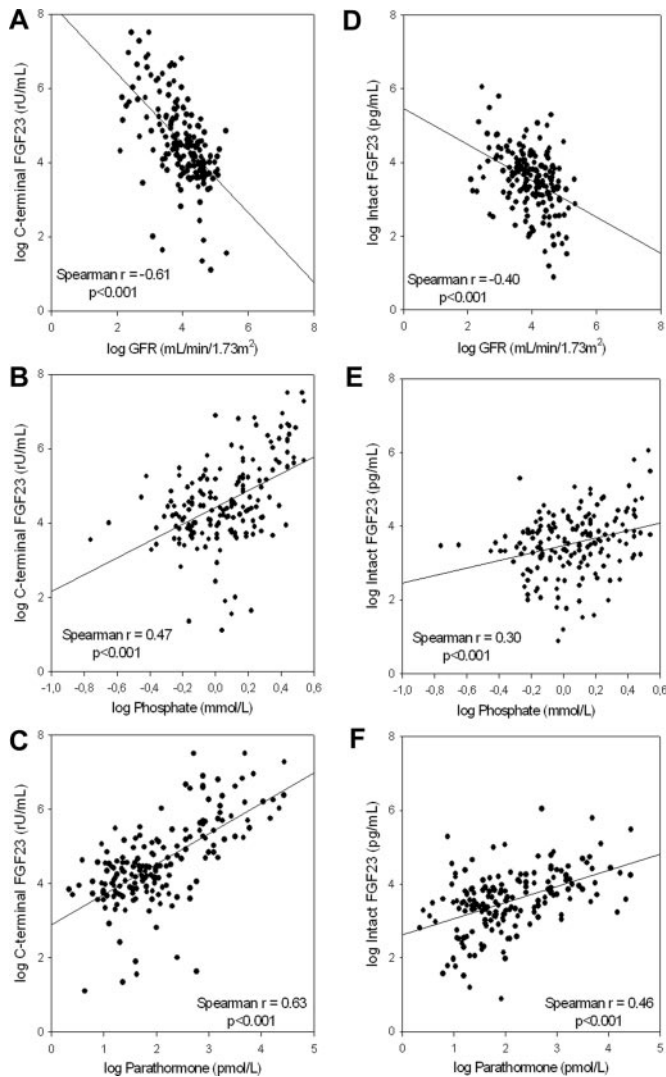
<sup>b</sup>P values are for comparison across all four groups obtained from the Kruskal-Wallis test, one-way ANOVA, and  $\chi^2$  test, as appropriate.

**DISCUSSION**

The results of this prospective long-term study in a sizable cohort of white patients with nondiabetic CKD have identified FGF23 as a novel risk marker for the progression of CKD. Remarkably, apart from baseline GFR, FGF23 was the only independent predictor of progression among several parameters of the calcium-phosphate metabolism assessed. Moreover, both the c-terminal fragment and the biologically active intact FGF23 were independent predictors of progression. This finding is of particular interest because it points to a role of FGF23 in CKD progression apart from the problem of accumulation as a result of reduced renal clearance, which could be one potential explanation for elevated concentrations of c-terminal FGF23.

FGF23 is a recently identified “phosphatonin” that is thought to be implicated in the systemic balance of phosphate maintained by the interaction of intestine, bone, and kidneys.<sup>9–11</sup> In several clinical conditions, excessive activity of FGF23 resulted in hypophosphatemia, low 1,25-OH<sub>2</sub>D<sub>3</sub> levels, and osteomalacia.<sup>12,13</sup> Administration of recombinant FGF23 to experimental animals or overexpression of the FGF23 gene *in vivo* produced similar derangements of calcium-phosphate metabolism, whereas inactivation of this gene caused hyperphosphatemia and high circulating 1,25-OH<sub>2</sub>D<sub>3</sub> levels.<sup>14–16</sup> The physiologic stimulus for FGF23 secretion seems to be hyperphosphatemia caused by a dietary phosphate load.<sup>17</sup> The increase in FGF23 levels in response to dietary phosphate promotes phosphaturia and suppresses renal production of active vitamin D. However, in the presence of progressive CKD, serum FGF23 levels increase in parallel with the deterioration of renal function and the increase of serum phosphate and PTH concentrations.<sup>9,18–21</sup> In predialysis patients and in patients who were on maintenance hemodialysis, high FGF23 serum levels were correlated with those of phosphate, pointing to a disrupted feedback loop resulting in very high levels of serum FGF23. We could clearly confirm these findings in our study population with mild to moderate CKD, in which the decrease of renal function across a wide range of GFR was paralleled by a significant and marked increase particularly of c-terminal but also of intact FGF23 blood concentrations (Table 1, Figure 1). However, because we measured only baseline serum phosphate, calcium, PTH, and FGF23 levels in our cross-sectional study, we cannot distinguish whether the hormone levels are the cause of abnormal serum calcium and phosphate levels or their consequence.

In experimental studies, the increase of FGF23 levels preceded the decrease of serum 1,25-OH<sub>2</sub>D<sub>3</sub> concentrations, suggesting an important role of FGF23 in the development of secondary hyperparathyroidism of patients with CKD. This point is further corroborated by results of recent experimental<sup>22</sup> and clinical studies.<sup>21</sup> For example, Gutierrez *et al.*<sup>21</sup> found an increase in FGF23 serum levels in early stages of CKD, even before serum phosphate and calcium concentrations had become abnormal. The authors concluded that increased FGF23 levels



**Figure 1.** Correlation analysis of c-terminal (A through C) as well as intact fibroblast growth factor 23 (FGF23; D through F) with GFR, serum phosphate, and parathormone. All parameters have been analyzed based on the log scale.

are presumably a central factor in the early pathogenesis of secondary hyperparathyroidism. Collectively, these findings implicate that circulating FGF23 is a physiologic regulator of phosphate balance and as such also a potential uremic toxin.<sup>10,11</sup> An alternative interpretation of our findings is that FGF23 levels represent the overall burden of phosphate loading above and beyond that represented by a single measurement of fasting serum phosphate. At any rate, FGF23 is an excellent indicator of the complex derangements of calcium-phosphate metabolism induced by CKD and probably also a valuable surrogate parameter to indicate more distal sequelae of the deranged mineral metabolism. This may be clinically important: It has been known for decades that in ESRD, hyperphosphatemia causes soft tissue calcification including vascular calcification.<sup>23</sup> However, until recently, its impact on survival in patients with CKD had not been appreciated. In 1998

Block *et al.*<sup>24</sup> found that in dialysis patients, survival was significantly less when the predialysis serum phosphate concentration exceeded 6.5 mg/dl. This increase was related to death from coronary heart disease, possibly as a result of accelerated coronary plaque calcification.<sup>25</sup> Phosphate—more specific, intracellular phosphate—plays a major role in the genesis of vascular calcification, particularly in the presence of ionized calcium.<sup>26</sup> However, the adverse role of high serum phosphate concentrations is not restricted to patients with CKD,<sup>27</sup> and even in nonrenal patients, serum phosphate concentrations were positively and significantly correlated with the severity of coronary artery disease and with the severity of coronary artery stenoses and presence of occlusions.<sup>28</sup> In a *post hoc* analysis of the Cholesterol and Recurrent Events (CARE) study in 4127 patients, Tonelli *et al.*<sup>29</sup> found that serum phosphate levels (even within the upper normal range) were associated with more adverse cardiovascular outcomes. They also found a direct association between GFR and serum phosphate concentrations, but the relation between phosphate and outcome still persisted when individuals with a GFR <60 ml/min were excluded from the analysis. It is interesting that the calcium-phosphate product was not independently associated with adverse outcome.<sup>29</sup> Taken together, these data suggest that even minor derangements of calcium-phosphate metabolism, particularly of serum phosphate levels, may contribute to cardiovascular complications in nonrenal patients as well as in patients with CKD.

This study points to an additional potential consequence, namely that serum phosphate levels have an impact on progression of renal disease. The experimental work of Haut and Alfrey and colleagues<sup>30,31</sup> showed that renal damage was aggravated by phosphate loads. To our knowledge, our observation is the first large prospective study to investigate the influence of changes in calcium-phosphate metabolism and the role of FGF23 on progression of CKD. Given the physiologic role of FGF23 in phosphate metabolism, this “phosphatonin” may turn out to be an excellent indicator of cardiovascular risk even in patients without CKD. Further studies on this issue are warranted.

The cohort investigated in our study comprised relatively young patients with mostly mild to moderate impairment of renal function or even normal GFR at the baseline examination. The remarkable power of baseline GFR as a predictor of progression highlights the importance of impaired renal function as a key determinant of progressive renal damage. In addition, this finding may explain why more patients in the “progressor” subgroup received vitamin D and phosphate binders, because derangements of calcium-phosphate metabolism become more evident with advanced CKD. In our patients, FGF23 levels increased with decreasing GFR, but it is important to point out that the impressive prediction of progression by both c-terminal and intact FGF23 is already adjusted for GFR. We have to admit that the measurement of GFR even with iohexol clearance may not be perfect and also does not reflect all aspects of kidney

**Table 2.** Baseline clinical and laboratory data of 177 patients with completed follow-up with further stratification of those with and without progression during the follow-up period<sup>a</sup>

Parameter	Nonprogressors (n = 112)	Progressors (n = 65)	P
Gender (male/female; n [%])	74/38 (66/34)	44/21 (68/32)	0.83
Age (yr)	44.8 ± 12.6	49.1 ± 11.1	0.03
Body mass index (kg/m <sup>2</sup> )	24.8 ± 3.5	25.7 ± 3.9	0.13
Current smokers (n [%])	18 (16)	16 (25)	0.21
Systolic BP (mmHg)	136 ± 22	137 ± 17	0.72
Diastolic BP (mmHg)	86 ± 14	88 ± 12	0.34
Serum creatinine (mg/dl)	1.54 ± 0.61 (1.14, 1.40, 1.80)	3.21 ± 1.31 (2.21, 3.10, 3.94)	<0.001
GFR (ml/min per 1.73m <sup>2</sup> )	79 ± 38 (50, 74, 99)	38 ± 25 (20, 33, 46)	<0.001
Proteinuria (g/24 h per 1.73 m <sup>2</sup> )	0.87 ± 0.95 (0.14, 0.46, 1.25)	1.25 ± 0.83 (0.61, 1.09, 1.78)	<0.001
Serum albumin (g/dl)	4.57 ± 0.43	4.53 ± 0.36	0.50
hsCRP (mg/L)	0.28 ± 0.32	0.29 ± 0.31	0.65
FGF23 c-terminal (rU/ml)	92 ± 113 (41, 64, 96)	351 ± 394 (96, 190, 492)	<0.001
FGF23 intact (pg/ml)	35 ± 28 (18, 29, 45)	69 ± 70 (31, 48, 80)	<0.001
Calcium (mmol/L)	2.38 ± 0.22 (2.30, 2.37, 2.44)	2.32 ± 0.17 (2.24, 2.31, 2.43)	0.04
Phosphate (mmol/L)	1.04 ± 0.38 (0.86, 1.01, 1.14)	1.25 ± 0.27 (1.02, 1.23, 1.49)	<0.001
Ca × P product (mmol <sup>2</sup> /L <sup>2</sup> )	2.46 ± 0.85 (2.00, 2.37, 2.76)	2.90 ± 0.65 (2.43, 2.83, 3.22)	<0.001
Parathormone (pmol/L)	6.5 ± 5.3 (3.4, 5.0, 7.2)	22.5 ± 20.0 (8.0, 16.0, 29.5)	<0.001
Use of vitamin D (n [%])	9 (8)	20 (31)	<0.001
Use of a phosphate binder (n [%])	2 (2)	10 (15)	0.001

<sup>a</sup>Data are means ± SD and 25th, 50th (median), and 75th percentiles for skewed variables where appropriate.

dysfunction. Therefore, we cannot exclude the possibility that the prediction of progression by intact FGF23 might also result from commutation of biologically inert degradation products reflecting residual confounding by severity of kidney function. However, the estimates of CKD progression by FGF23 were still highly significant after adjustment for GFR. This finding suggests that FGF23 is not simply a surrogate marker for baseline GFR and that FGF23 is a marker on its own providing prediction beyond the information obtained by the measurement of GFR.

In our cohort, systolic and diastolic BP at baseline were comparable in patients who progressed to a renal end point during follow-up and those who did not. The former needed more aggressive antihypertensive treatment, however, to

achieve the same level of BP control. We emphasize that the use of angiotensin-converting enzyme inhibitors was comparable between groups. It is likely that identification of further progression predictors and potentially progression promoters such as FGF23 was facilitated by the almost equal BP control in progressors and nonprogressors.

The exclusion criteria in this study yielded a selected group of patients. Further studies must show whether the association between FGF23 and progression can be found also in other types of CKD, such as diabetic nephropathy, or nephrotic forms of kidney disease. The study had a follow-up time of adequate duration, and the primary end points were reached by one third of the participants. Therefore, the data are sufficiently solid to draw firm conclusions for the entire cohort,

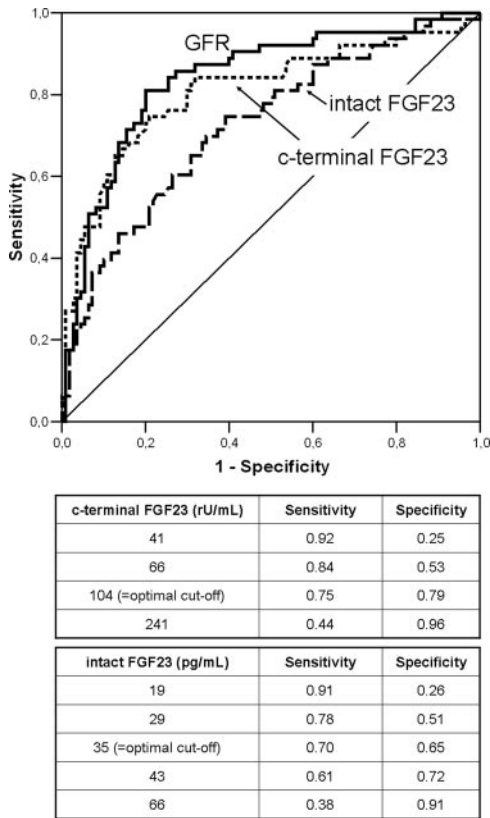
**Table 3.** Association of baseline variables with progression of kidney disease during the observation period using multiple Cox proportional hazards regression models<sup>a</sup>

Parameter Variable (increment)	Model 1 Adjusted for Age and Gender		Model 2 Adjusted for Age, Gender, and All Other Variables of This Table		Model 3 Adjusted for Age, Gender, and All Other Variables Excluding FGF23	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
GFR (10 ml/min per 1.73 m <sup>2</sup> )	0.668 (0.590 to 0.757)	<0.001	0.762 (0.666 to 0.872)	<0.001	0.736 (0.646 to 0.837)	<0.001
Proteinuria (1 g/24 h)	1.298 (1.023 to 1.647)	0.032	1.244 (0.921 to 1.679)	0.154	1.142 (0.862 to 1.515)	0.354
Calcium (0.1 mmol/L)	0.885 (0.746 to 1.049)	0.159	1.005 (0.877 to 1.153)	0.941	1.038 (0.925 to 1.164)	0.525
Phosphate (0.1 mmol/L)	1.091 (1.049 to 1.134)	<0.001	1.052 (0.952 to 1.162)	0.322	1.090 (0.995 to 1.194)	0.063
Ca × P (0.1 mmol <sup>2</sup> /L <sup>2</sup> )	1.041 (1.022 to 1.061)	<0.001	1.024 (0.981 to 1.068) <sup>b</sup>	0.279	1.041 (1.002 to 1.082) <sup>b</sup>	0.040
Parathormone (1 pmol/L)	1.041 (1.029 to 1.052)	<0.001	1.009 (0.993 to 1.025)	0.273	1.019 (1.005 to 1.033)	0.008
FGF23 c-terminal (10 rU/ml)	1.028 (1.021 to 1.034)	<0.001	1.014 (1.005 to 1.024) <sup>c</sup>	0.002		
FGF23 intact (10 pg/ml)	1.111 (1.075 to 1.149)	<0.001	1.061 (1.018 to 1.106) <sup>c</sup>	0.005		

<sup>a</sup>CI, confidence interval; HR, hazard ratio.

<sup>b</sup>Because Ca × P is the product term of the variable calcium and phosphate, estimates for Ca × P were based on a model that did not include calcium and phosphate at the same time. In model 2, the estimate for Ca × P was adjusted for age, gender, GFR, proteinuria, parathormone, and c-terminal FGF23.

<sup>c</sup>C-terminal FGF23 and intact FGF23 were not included at the same time but were adjusted for the same variables: Age, gender, GFR, proteinuria, calcium, phosphate, and parathormone.



**Figure 2.** Receiver operating characteristics (ROC) curve of GFR and plasma c-terminal and intact FGF23 concentrations with progression of kidney disease as status variable. The area under the curve (AUC) for GFR, c-terminal, and intact FGF23 are 0.84, 0.81, and 0.72, respectively. The inserted tables present sensitivity and specificity of the four cutoffs derived from the quintile thresholds for c-terminal and intact FGF23, respectively. For c-terminal FGF23, the threshold between the third and fourth quintiles was equal to the ROC-derived optimal cutoff. For intact FGF23, the optimal cutoff is presented additionally.

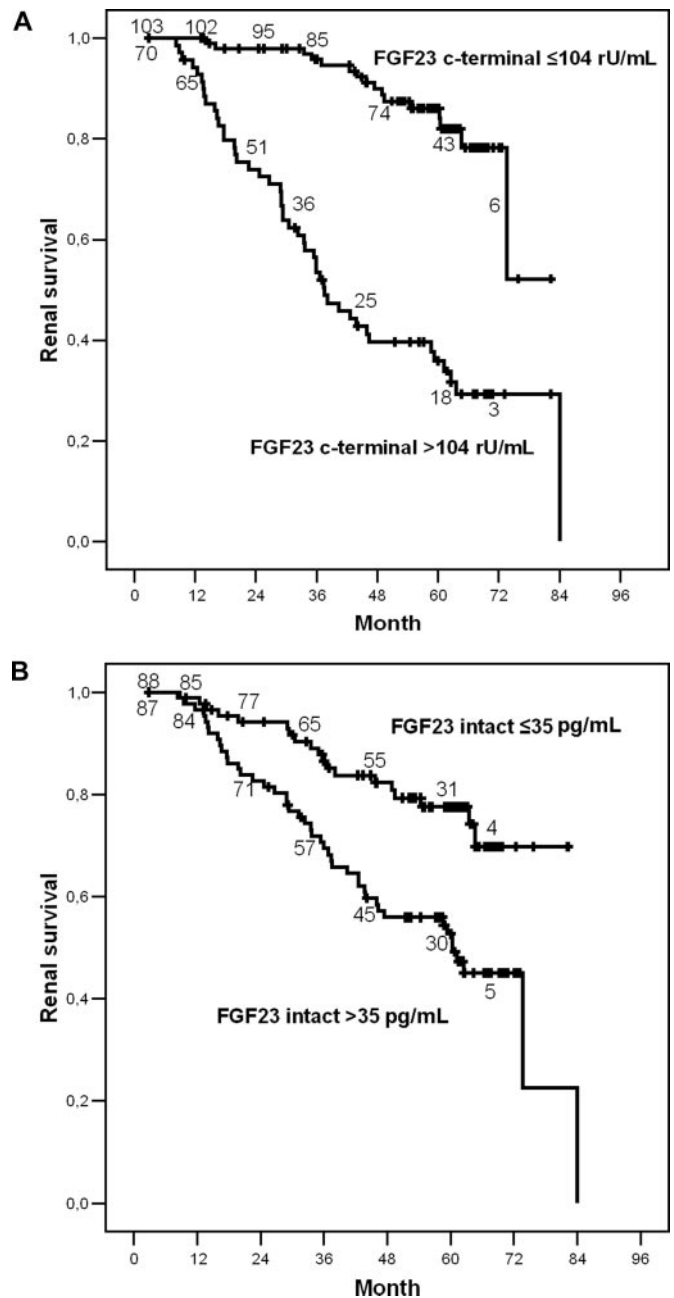
despite a considerable loss during follow-up of participants who had superior kidney function at baseline when compared with the followed patients. An additional limitation is the lack of data on phosphate intake in our patients.

In this prospective study comprising a cohort of patients with primary CKD, we identified intact FGF23 and, additionally, the easier-to-measure c-terminal FGF23 fragment as novel predictors of CKD progression. Given the physiologic role of FGF23 in calcium-phosphate metabolism, it may also serve as a clinically useful indicator of early alterations of calcium-phosphate metabolism, correction of which might modify progression of CKD<sup>31</sup> and possibly also cardiovascular risk.<sup>29</sup>

**CONCISE METHODS**

**Patients and Baseline Investigations**

We examined at baseline 227 white patients who were between 18 and 65 yr of age and had nondiabetic CKD and various degrees of



**Figure 3.** Kaplan-Meier curves of renal end points in patients with below and above optimal cutoff of plasma c-terminal (A) FGF23 concentrations and for intact (B) FGF23 concentrations below and above the median. In patients with c-terminal FGF23 levels above the optimal cutoff (>104 rU/ml), progression was significantly faster (log-rank test,  $P < 0.0001$ ); the same was observed in patients with intact FGF23 levels above the median (35 pg/ml; log-rank test,  $P = 0.0004$ ). Numbers near the survival curves represent the number of patients at risk with FGF23 levels below and above the optimal cutoff at the times 0, 12, 24, 36, 48, 60, and 72 mo.

renal impairment. These patients were recruited from eight nephrology departments in Germany, Austria, and South Tyrol as described previously.<sup>32</sup> The study was approved by the institu-

tional ethic committees, and all participants gave written informed consent. They had stable renal function for at least 3 mo before entry into the study. Exclusion criteria were treatment with immunosuppressive agents, fish oil, or erythropoietin; serum creatinine  $>6$  mg/dl; diabetes of any type; malignancy; liver, thyroid, or infectious disease; nephrotic syndrome (defined as proteinuria  $>3.5$  g/1.73 m<sup>2</sup> per d); organ transplantation; allergy to ionic contrast media; and pregnancy. According to the NKF classification of CKD, our study cohort showed the following stages of CKD: GFR  $\geq 90$  ml/min per 1.73 m<sup>2</sup> (stage 1) in 72 (31.7%) patients, GFR 60 to 89 ml/min per 1.73 m<sup>2</sup> (stage 2) in 49 (21.6%) patients, GFR 30 to 59 ml/min per 1.73 m<sup>2</sup> (stage 3) in 63 (27.8%) patients, and GFR  $<30$  ml/min per 1.73 m<sup>2</sup> (stages 4 and 5) in 43 (18.9%) patients. The primary cause of kidney disease was glomerulonephritis in 97 (biopsy-confirmed in 90) patients, adult polycystic kidney disease in 37 patients, interstitial nephritis in 24 patients, other types of kidney disease in 43 patients, and unknown in 26 patients.

For avoiding interobserver differences, all patients were recruited by one physician who visited all participating centers. Patient history, including smoking habits and antihypertensive treatment at baseline, was recorded by interview and confirmed by checking patient records. This was complemented by clinical examination, including assessment of body mass index and BP. Hypertension was defined as BP  $>140/90$  mmHg and/or the use of antihypertensive medication. Antihypertensive medication was withheld on the day of the study to minimize interference with measurements of the GFR. Antihypertensive drugs were taken by 179 (79%) patients: Diuretics ( $n = 83$ ; 37%), angiotensin-converting enzyme inhibitors ( $n = 123$ ; 54%), calcium channel blockers ( $n = 78$ ; 34%),  $\beta$  receptor blockers ( $n = 67$ ; 30%), and  $\alpha$ -1 receptor blockers ( $n = 36$ ; 16%).

### Prospective Follow-Up

After the baseline investigation, patients were followed prospectively until the primary study end point or the end of the observation period was reached. The primary end point was defined as doubling of baseline serum creatinine and/or terminal renal failure necessitating renal replacement therapy. A total of 177 (78%) patients from the baseline cohort could be assessed during the follow-up. Patients who were lost to follow-up ( $n = 50$ ) had at baseline a significantly better renal function than patients who were not lost for follow-up (*i.e.*, a higher mean GFR [ $91 \pm 44$  versus  $64 \pm 39$  ml/min per 1.73 m<sup>2</sup>;  $P < 0.01$ ]). However, both groups did not differ significantly with respect to age and gender. Patients who were lost to follow-up had moved away or were not referred by their physicians for follow-up visits in the renal units.

### Laboratory Measurements

Blood samples for measurement of routine chemistry, hsCRP, PTH, and c-terminal and intact FGF23 were taken after an overnight fast of at least 12 h. The samples were immediately centrifuged at  $1500 \times g$  and  $4^{\circ}\text{C}$  for 10 min, and the supernatants were stored in aliquots at  $-80^{\circ}\text{C}$  until further use. PTH was measured with an immunoradiometric assay, and FGF23 was measured using

both the human c-terminal and the intact ELISA (Immutopics, San Clemente, CA).<sup>10</sup> The interassay coefficients of variability of c-terminal FGF23 are 6.5% at 40 rU/ml and 7.5% at 175 rU/ml, respectively, with a lower detection limit of 3.0 rU/ml. For intact FGF23, the interassay coefficients of variability are 6.1% at 15.6 pg/ml and 6.5% at 166 pg/ml, respectively, with a lower detection limit of 1.0 pg/ml. Measurements of routine chemistry including hsCRP were performed with routine laboratory tests. In addition, GFR was assessed in all patients using the iothexol clearance technique as described in detail elsewhere.<sup>33</sup>

### Statistical Analyses

Statistical analysis was performed with the SPSS for Windows 12.01 (SPSS, Chicago, IL). Univariate comparisons of continuous variables between various groups were performed using an unpaired *t* test or the nonparametric Wilcoxon rank sum test in case of non-normally distributed variables. Dichotomized variables were compared using Pearson  $\chi^2$  test. All statistical tests were performed at the two-sided 0.05 level of significance. Data are presented as means  $\pm$  SD and as median and 25th and 75th percentiles for skewed variables where appropriate. Univariate correlation analysis was performed by Spearman correlation analysis. For calculation of ROC curves for the predictor variables c-terminal and intact FGF23, patients were stratified into two groups (those who progressed and those who did not over the study period), and for each cutoff value *c*, sensitivity was defined as the proportion of progressors with predictor variables above *c* and specificity as the proportion of nonprogressors with values below *c*. The optimal cutoff value for the predictor variables was defined as the cutoff obtaining the highest sum of sensitivity and specificity. Calculation of the AUC and a test of the null hypothesis that it equals 50% (equivalent to a random 50:50 prognosis) were performed using the Mann-Whitney test. Kaplan-Meier time-to-event curves were generated for patients with c-terminal and intact FGF23 concentrations above and below optimal cutoff levels. Adjusted risk estimates for progression end points were calculated using multivariable Cox proportional hazards regression analysis. Because  $\text{Ca} \times \text{P}$  is the product term of the variable calcium and phosphate, estimates of  $\text{Ca} \times \text{P}$  were based on a model that did not include calcium and phosphate at the same time, to avoid multicollinearity. Similarly, intact and c-terminal FGF23 were not offered at the same time in a particular model. Exploratory graphical analysis and tests of specific violations indicated no departure from the assumption of proportional hazards.

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## DISCLOSURES

None.

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