Elevated Fibroblast Growth Factor 23 is a Risk Factor for Kidney Transplant Loss and Mortality

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ABSTRACT

An increased circulating level of fibroblast growth factor 23 (FGF23) is an independent risk factor for mortality, cardiovascular disease, and progression of chronic kidney disease (CKD), but its role in transplant allograft and patient survival is unknown. We tested the hypothesis that increased FGF23 is an independent risk factor for all-cause mortality and allograft loss in a prospective cohort of 984 stable kidney transplant recipients. At enrollment, estimated GFR (eGFR) was 51 $\pm$ 21 ml/min per 1.73 m^2 and median C-terminal FGF23 was 28 RU/ml (interquartile range, 20 to 43 RU/ml). Higher FGF23 levels independently associated with increased risk of the composite outcome of all-cause mortality and allograft loss (full model hazard ratio: 1.46 per SD increase in logFGF23, 95% confidence interval: 1.28 to 1.68, $P < 0.001$). The results were similar for each component of the composite outcome and in all sensitivity analyses, including prespecified analyses of patients with baseline eGFR of 30 to 90 ml/min per 1.73 m^2. In contrast, other measures of phosphorus metabolism, including serum phosphate and parathyroid hormone (PTH) levels, did not consistently associate with outcomes. We conclude that a high (or elevated) FGF23 is an independent risk factor for death and allograft loss in kidney transplant recipients.

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Kidney transplantation is the preferred treatment for the growing number of patients with ESRD. A successful kidney transplant restores renal function to near normal, frees patients from the rigors of a relentless dialysis schedule, and dramatically prolongs survival.[1] Allograft and patient survival have improved steadily over past decades as a result of advances in operative techniques, immune suppression regimens, and prophylaxis against opportunistic infections.[2] As a result, death and disability caused by cardiovascular disease and late graft loss caused by chronic allograft nephropathy have surpassed infection and early allograft loss caused by rejection as the primary threats to the health of kidney transplant recipients.[3]

Disordered phosphorus metabolism is a common complication of chronic kidney disease (CKD)

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that is associated with adverse cardiovascular and renal outcomes. Increased circulating levels of fibroblast growth factor 23 (FGF23), phosphate, and parathyroid hormone (PTH) are independent risk factors for kidney disease progression, cardiovascular disease, and mortality in dialysis, predialysis CKD, and non-CKD patients.[4–14] Many kidney transplant recipients develop hypophosphatemia and hypercalcemia because of persistent elevations of FGF23 and PTH in the early period after a successful transplant.[15,16] Although these overt changes in serum phosphate and calcium usually resolve without intervention within weeks to months,[17] the impact on long-term patient and allograft survival of increased FGF23 and PTH levels is unknown. We performed a prospective study of 984 prevalent kidney transplant recipients to test the hypothesis that increased FGF23 is an independent risk factor for future mortality and allograft loss and that increased FGF23 is more strongly associated with adverse outcomes than increased PTH or phosphate levels.

Table 1. Baseline characteristics of the overall study population and according to tertiles of FGF23

	FGF23 (RU/ml)				
	All Patients (*n* = 984) 28 (20, 43)	**FGF23 Tertile 1 (*n* = 328) 18 (15, 20)**	**FGF23 Tertile 2 (*n* = 328) 28 (26, 32)**	**FGF23 Tertile 3 (*n* = 328) 55 (43, 86)**	***P*[a]**
Age, years	51 ± 13	49 ± 13	50 ± 13	54 ± 12	<0.01
Male (%)	57	56	59	56	0.6
Etiology of kidney disease (%)					0.3
glomerulonephritis	23	23	25	20	
tubulointerstitial nephritis	13	15	12	13	
polycystic kidney disease	18	15	16	23	
diabetes	4	6	5	3	
hypertension	7	6	7	6	
other or unknown	35	35	35	35	
Dialysis duration, months	20 (9, 38)	19 (9, 41)	19 (9, 39)	21 (9, 36)	1.0
Deceased donor transplant (%)	96	96	95	97	0.6
Delayed graft function (%)	26	24	24	30	0.1
History of acute rejection (%)	35	32	32	40	0.05
Transplant vintage, months	72 (40, 114)	62 (35, 103)	66 (35, 107)	91 (51, 127)	<0.01
Body mass index, kg/m^2	27 ± 5	27 ± 5	27 ± 5	26 ± 5	<0.01
Systolic BP, mmHg	142 ± 19	140 ± 19	140 ± 18	145 ± 20	<0.01
Diastolic BP, mmHg	84 ± 12	84 ± 12	83 ± 12	85 ± 13	0.4
Current smoker (%)	19	17	20	18	0.5
Charlson comorbidity index	2 (2, 4)	2 (2, 3)	2 (2, 3)	2 (2, 4)	0.02
Laboratory results					
creatinine, mg/dl	1.4 (1.2, 1.8)	1.2 (1.0, 1.5)	1.4 (1.1, 1.7)	1.8 (1.4, 2.3)	<0.01
eGFR, ml/min per 1.73 m^2	51 ± 21	61 ± 19	53 ± 19	39 ± 19	<0.01
eGFR > 60 ml/min per 1.73 m^2	32	50	32	13	<0.01
eGFR 30 to 60 ml/min per 1.73 m^2	51	47	56	50	<0.01
eGFR < 30 ml/min per 1.73 m^2	17	3	12	37	<0.01
albumin, g/dl	4.0 ± 0.4	4.1 ± 0.4	4.1 ± 0.4	3.9 ± 0.5	<0.01
calcium, mg/dl	9.4 ± 0.6	9.5 ± 0.5	9.5 ± 0.6	9.4 ± 0.8	0.01
phosphate, mg/dl	3.3 ± 0.9	3.2 ± 0.9	3.2 ± 0.6	3.6 ± 1.0	<0.01
25-hydroxyvitamin D, ng/ml	11.2 ± 7.6	11.9 ± 7.9	11.1 ± 7.6	10.5 ± 7.2	0.04
parathyroid hormone, pg/ml	68 (48, 103)	59 (43, 83)	70 (50, 101)	85 (54, 150)	<0.01
hemoglobin, g/dl	13.5 ± 1.7	13.7 ± 1.4	13.7 ± 1.6	13.0 ± 1.9	<0.01
white blood cells (cells × 10^3)	7.9 ± 2.3	7.9 ± 2.2	7.8 ± 2.2	7.9 ± 2.6	0.6
C-reactive protein (mg/L)	3.1 (1.5, 6.8)	2.9 (1.5, 6.7)	3.1 (1.5, 7.2)	3.6 (1.4, 6.7)	0.7
FGF23-modifying therapies					
1,25-dihydroxyvitamin D (%)	33	28	32	40	<0.01
dietary phosphate binders (%)	6	4	4	9	<0.01
Immune suppression agents (%)					
steroids	81	75	81	88	<0.01
calcineurin inhibitor	90	91	91	89	0.7
mycophenolate mofetil	78	82	81	71	0.01
sirolimus	8	9	7	7	0.6

Values reported as %, means ± SD, or median (interquartile range) as appropriate.
[a]*P* represents tests of significance from one-way ANOVA, Kruskal-Wallis, or χ^2 tests.

RESULTS

Characteristics of the Study Population

Baseline characteristics of the study population are presented in Table 1. Overall, median FGF23 levels were in the normal range and were lower than in previous reports of CKD patients with comparable estimated GFR (eGFR).[18] Compared with the lower tertiles, patients in the highest FGF23 tertile were older, had a longer transplant vintage, had lower eGFR, had lower serum albumin, calcium, and hemoglobin concentrations, had higher serum phosphate and PTH concentrations, and were more likely to have a history of prior rejection and current treatment with 1,25-dihydroxyvitamin D, dietary phosphate binders, and steroids. FGF23 ($r = -0.46$), PTH ($r = -0.31$), serum phosphate ($r = -0.38$), and hemoglobin ($r = 0.45$) correlated significantly with eGFR, whereas FGF23 ($r = 0.17$) and hemoglobin ($r = -0.10$) correlated significantly with transplant vintage.

FGF23 and Clinical Outcomes

During a median follow-up of 37 months (interquartile range, 35 to 39 months), 87 patients died (31.7/1000 patient-years of follow-up) and 101 patients had allograft loss (36.8/1000 patient-years of follow-up). These results are comparable to USRDS data for years 6 to 9 after transplant.[19] Univariate cumulative incidence plots for mortality, allograft loss, and their composite according to tertiles of FGF23 are presented in Figure 1. Increasing natural logFGF23 and ascending tertiles of FGF23 were independently associated with the composite endpoint in all multivariable analyses of the overall population, the prespecified subgroup of recipients with an eGFR of 30 to 90 ml/min per 1.73 m^2 whose future risk of events were least clear clinically at baseline (Table 2), and in models that stratified by serum phosphate (Table 3). The results were unchanged in analyses that adjusted for therapy with oral 1,25-dihydroxyvitamin D and phosphate binders, donor type, history of rejection or delayed graft function, immune suppression regimen, C-reactive protein levels, white blood cell count, and diabetes, and in models that stratified by tertiles of transplant vintage (data not shown). LogFGF23 and ascending tertiles of FGF23 were also independently associated with all-cause mortality and death-censored allograft loss in separate competing risks regression models (Table 4).

Comparative Analyses of PTH, Phosphate, and Hemoglobin

Although increased PTH was associated with increased risk of the composite endpoint in the univariate analysis of the overall population, adjusting for eGFR completely attenuated the effect (Table 5). There was no significant relationship between PTH and outcomes in any other multivariable model of the overall population or any model of the subgroup of recipients with eGFR of 30 to 90 ml/min per 1.73 m^2 (Table 5).

When analyzed on a continuous scale, increased serum phosphate was independently associated with the composite endpoint in the overall population, but there was no consistent association between phosphate tertiles and outcomes in adjusted analyses (Table 6). Furthermore, there was no associa-

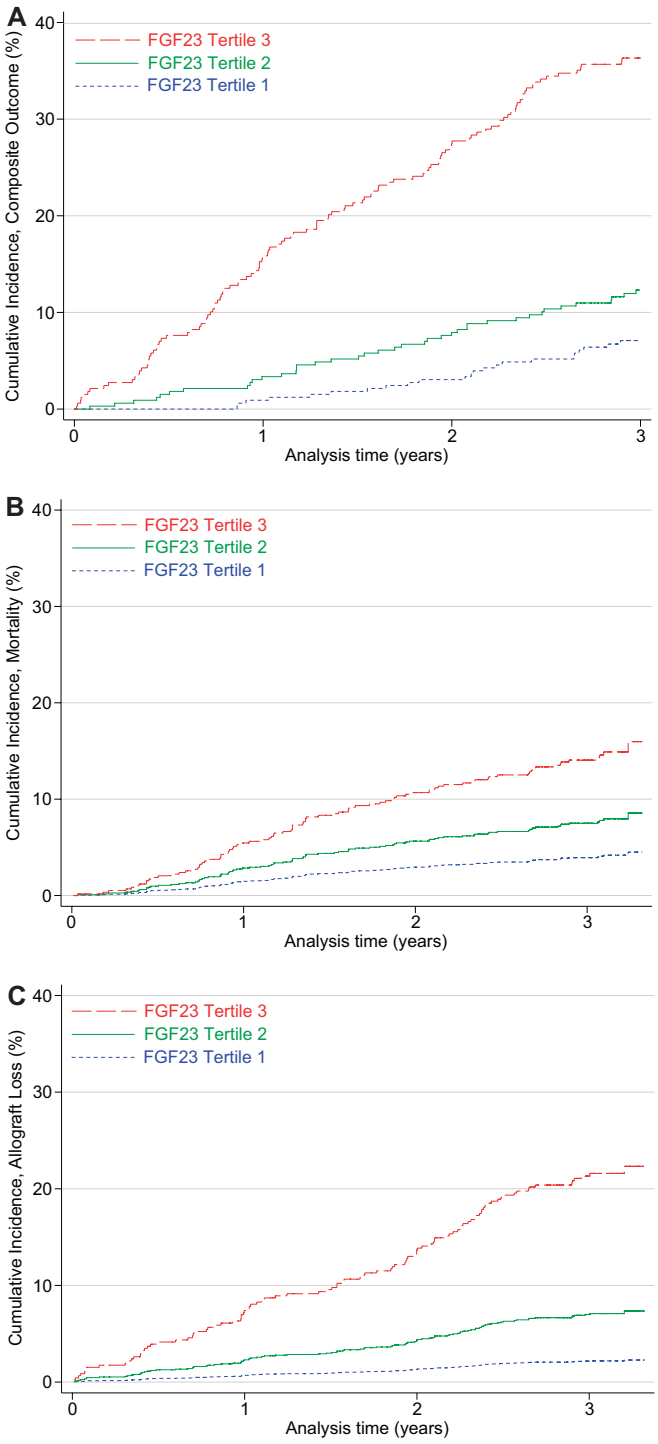

Figure 1. Higher FGF23 levels associate with greater incidence of mortality and allograft loss in kidney transplant recipients. Cumulative incidence plots of FGF23 tertiles and the composite outcome of all-cause mortality and allograft loss (A), all-cause mortality alone (B), and allograft loss alone (C). For each analysis, the *P* for trend was <0.0001.

Table 2. Hazard ratios of the composite outcome of death or allograft loss according to natural log-transformed FGF23 (per 1 SD increase) and ascending tertiles of FGF23

	Natural log FGF23		FGF23 Tertiles			
	per 1 SD	*P*	1	2	3	*P*[a]
All patients, $n = 984$						
crude	1.94	<0.001	Reference	1.96	6.49	<0.001
	1.77, 2.13			1.19, 3.25	4.15, 10.1	
eGFR	1.61	<0.001	Reference	1.40	3.04	<0.001
	1.43, 1.82			0.84, 2.33	1.89, 4.9	
eGFR, age, gender	1.62	<0.001	Reference	1.34	2.84	<0.001
	1.43, 1.84			0.80, 2.24	1.76, 4.57	
full model	1.46	<0.001	Reference	1.39	2.27	<0.001
	1.28, 1.68			0.83, 2.33	1.38, 3.72	
full model + phosphate	1.42	<0.001	Reference	1.42	2.23	0.001
	1.23, 1.62			0.85, 2.39	1.35, 3.67	
full model + logPTH	1.44	<0.001	Reference	1.39	2.23	0.001
	1.25, 1.65			0.83, 2.33	1.35, 3.66	
Restricted to patients with eGFR 30 to 90 ml/min per 1.73 m^2, $n = 765$						
crude	1.63	<0.001	Reference	1.75	3.96	<0.001
	1.37, 1.94			0.99, 3.12	2.32, 6.77	
eGFR	1.57	<0.001	Reference	1.55	2.92	<0.001
	1.29, 1.92			0.87, 2.77	1.67, 5.05	
eGFR, age, gender	1.55	<0.001	Reference	1.51	2.61	<0.001
	1.26, 1.90			0.85, 2.70	1.50, 4.54	
full model	1.43	0.002	Reference	1.57	2.24	0.005
	1.14, 1.78			0.87, 2.80	1.27, 3.94	
full model + phosphate	1.45	0.001	Reference	1.57	2.29	0.004
	1.17, 1.81			0.88, 2.81	1.30, 4.03	
full model + logPTH	1.42	0.003	Reference	1.54	2.18	0.007
	1.13, 1.77			0.86, 2.76	1.23, 3.85	

Full model is adjusted for eGFR, age, gender, systolic BP, body mass index, albumin, calcium, Charlson score, and the duration after transplant at the time of enrollment.
[a]*P* for linear trend.

Table 3. Hazard ratio (95% CI) of the composite outcome of death or allograft loss according to 1 SD increase in natural log-transformed FGF23

	Phosphate Tertile 1 (<2.9 mg/dl)	Phosphate Tertile 2 (2.0 to 3.5 mg/dl)	Phosphate Tertile 3 (>3.5 mg/dl)
Crude	1.95 (1.34, 2.78)	1.85 (1.45, 2.35)	1.76 (1.57, 1.98)
	$P < 0.001$	$P < 0.001$	$P < 0.001$
eGFR-adjusted	1.78 (1.21, 2.59)	1.69 (1.30, 2.20)	1.40 (1.19, 1.65)
	$P = 0.003$	$P < 0.001$	$P < 0.001$
Fully-adjusted	1.67 (1.13, 2.47)	1.52 (1.12, 2.06)	1.37 (1.14, 1.66)
	$P = 0.009$	$P = 0.008$	$P = 0.001$

Full model is adjusted for eGFR, age, gender, systolic BP, body mass index, albumin, calcium, Charlson score, and the duration after transplant at the time of enrollment.

tion between serum phosphate or phosphate tertiles and the composite outcome in any analysis of the subgroup of recipients with eGFR of 30 to 90 ml/min per 1.73 m^2 (Table 6).

The results of analyses of hemoglobin and risk of adverse outcomes mirrored those of phosphate. Significant associations were observed exclusively in analyses of continuous hemoglobin levels and unadjusted analyses of hemoglobin tertiles in the overall population. The latter effect was completely attenuated when adjusted for eGFR, and there was no association between hemoglobin and the composite outcome in any analysis of the subgroup of recipients with eGFR of 30 to 90 ml/min per 1.73 m^2 (data not shown). Figure 2 summarizes the contrasting results for the different exposures and highlights the specificity of FGF23 as a risk factor.

DISCUSSION

In this prospective study of stable kidney transplant recipients, elevated FGF23 levels were independently associated with increased future risk of death and allograft loss. The results were consistent across the individual outcomes and their composite, were robust to multiple analytical strategies and sensitivity analyses, and were consistent even in patients with the lowest phosphate levels. In contrast, other measures of phosphorus metabolism including PTH and serum phosphate were only weakly associated with adverse outcomes, if at all, and adjusting for PTH and serum phosphate did not alter the magnitude or strength of association between higher FGF23 levels and outcomes. As the first report linking increased FGF23 levels to greater risk of mortality and allograft loss in kidney transplant recipients, these results extend the

Table 4. Competing risks regression analyses of all-cause mortality and allograft loss with natural log-transformed FGF23 (per 1 SD increase) and ascending tertiles of FGF23 as the primary exposure

	logFGF23		FGF23 Tertiles			
	Per 1 SD	*P*	1	2	3	*P*[a]
Outcome of all-cause mortality with allograft loss as competing risk						
all patients, *n* = 984						
eGFR	1.66	<0.001	Reference	1.51	2.92	<0.001
	1.33, 2.07			0.78, 2.94	1.57, 5.45	
eGFR, age, gender	1.52	<0.001	Reference	1.37	2.18	0.01
	1.27, 1.84			0.71, 2.64	1.13, 3.99	
restricted to patients with eGFR 30 to 90 ml/min per 1.73 m^2, *n* = 765						
eGFR	2.03	<0.001	Reference	1.52	3.05	0.004
	1.51, 2.73			0.68, 3.40	1.42, 6.57	
eGFR, age, gender	1.73	<0.001	Reference	1.35	2.00	0.08
	1.30, 2.29			0.61, 2.98	0.91, 4.41	
Outcome of allograft loss with all-cause mortality as competing risk						
all patients, *n* = 984						
eGFR	1.37	0.007	Reference	1.40	2.75	<0.001
	1.09, 1.71			0.64, 3.09	1.40, 5.38	
eGFR, age, gender	1.32	0.002	Reference	1.41	2.97	<0.001
	1.11, 1.57			0.64, 3.11	1.50, 5.88	
restricted to patients with eGFR 30 to 90 ml/min per 1.73 m^2, *n* = 765						
eGFR	1.38	0.06	Reference	1.56	2.48	0.01
	0.98, 1.93			0.69, 3.53	1.19, 5.18	
eGFR, age, gender	1.30	0.04	Reference	1.59	2.69	0.007
	1.01, 1.68			0.70, 3.60	1.28, 5.67	

[a]*P* for linear trend.

findings of previous studies of dialysis, predialysis, and non-CKD populations, in which increased FGF23 was strongly associated with cardiovascular disease, kidney disease progression, and mortality.[4–10]

FGF23 is secreted by osteocytes and regulates phosphorus homeostasis in conjunction with PTH and vitamin D.[20] Although FGF receptors (FGFRs) are widely expressed, FGF23 achieves its tissue specificity in the kidney and the parathyroids through high-affinity binding to FGFR via its co-receptor, klotho, the expression of which is concentrated in these organs.[21] FGF23 stimulates phosphaturia, inhibits PTH secretion, and lowers circulating levels of 1,25-dihydroxyvitamin D by inhibiting renal 1-α hydroxylase and stimulating the catabolic 24-hydroxylase.[20] In health, high phosphorus diets and 1,25-dihydroxyvitamin D stimulate FGF23 secretion, whereas low phosphorus diets lower FGF23 levels.[22] Through its effects on phosphate excretion, vitamin D, and PTH, FGF23 maintains serum phosphate levels within a narrow range despite fluctuation in dietary phosphorus intake.

FGF23 levels are constitutively elevated beginning in early stages of CKD, presumably to maintain normal phosphorus balance despite reduced excretory capacity.[23] Thus, the characteristic metabolic profile of patients with predialysis CKD is normal serum phosphate with increased FGF23 levels, increased fractional excretion of phosphate, and decreased levels of 1,25-dihydroxyvitamin D.[18] Once patients reach dialysis, levels of biologically intact FGF23 are markedly increased up to 1000 times the normal range.[24,25] The extreme secondary increase in FGF23 levels in dialysis patients sets the stage for alterations in phosphorus handling in the post-transplant period. High FGF23 contributes to early hypophosphatemia in the majority of kidney transplant recipients, persistent hypophosphatemia in some, and, along with PTH, subtle protracted reductions in serum phosphate and persistent elevations in urinary fractional excretion of phosphate compared with healthy individuals.[15–17] Until now, no studies examined whether FGF23 had an adverse impact on hard clinical outcomes in kidney transplant recipients.

Although FGF23, PTH, and serum phosphate are all physiologically interrelated, and each correlated significantly with eGFR, only FGF23 emerged as a robust independent risk factor for mortality and allograft loss. In contrast, increased serum phosphate was associated with adverse outcomes in fully adjusted analyses within the overall population only when it was expressed as a continuous variable, and it was not associated with adverse outcomes in any analysis of the subgroup of patients with intermediate eGFR. Furthermore, PTH and hemoglobin, which correlated with eGFR to a virtually identical extent as FGF23, were also not associated with adverse outcomes in any adjusted analysis. Thus, a critical finding of this study is the specificity of the results for FGF23 relative to other exposures, which argues strongly against significant confounding by reduced eGFR. In further support of a GFR-independent effect of FGF23,

Table 5. Hazard ratios of the composite outcome of death or allograft loss according to natural log-transformed PTH (per 1 SD increase) and ascending tertiles of PTH

	logPTH		PTH Tertiles			
	per 1 SD	*P*	1	2	3	*P*[a]
All patients, $n = 984$						
crude	1.35	<0.001	Reference	0.89	1.80	<0.001
	1.17, 1.57			0.60, 1.31	1.28, 2.54	
eGFR	1.00	0.99	Reference	0.87	0.96	0.87
	0.87, 1.14			0.58, 1.29	0.67, 1.37	
eGFR, age, gender	1.00	0.99	Reference	0.85	0.92	0.70
	0.87, 1.14			0.57, 1.26	0.64, 1.32	
full model	1.09	0.20	Reference	1.07	1.12	0.53
	0.95, 1.25			0.71, 1.61	0.78, 1.62	
full model + phosphate	1.09	0.19	Reference	1.09	1.15	0.45
	0.96, 1.25			0.72, 1.64	0.80, 1.67	
full model + logFGF23	1.07	0.29	Reference	1.10	1.16	0.43
	0.94, 1.22			0.73, 1.65	0.80, 1.69	
Restricted to patients with eGFR 30 to 90 ml/min per 1.73 m^2, $n = 765$						
crude	1.22	0.09	Reference	1.16	1.58	0.07
	0.97, 1.54			0.70, 1.92	0.96, 2.61	
eGFR	1.06	0.61	Reference	1.04	1.11	0.68
	0.84, 1.34			0.63, 1.72	0.66, 1.89	
eGFR, age, gender	1.06	0.60	Reference	1.02	1.08	0.76
	0.84, 1.35			0.61, 1.69	0.65, 1.81	
full model	1.18	0.18	Reference	1.24	1.35	0.26
	0.93, 1.50			0.73, 2.09	0.80, 2.30	
full model + phosphate	1.17	0.21	Reference	1.23	1.33	0.29
	0.92, 1.48			0.73, 2.08	0.78, 2.26	
full model + logFGF23	1.15	0.25	Reference	1.17	1.29	0.36
	0.90, 1.46			0.69, 1.98	0.75, 2.20	

Full model is adjusted for eGFR, age, gender, systolic BP, body mass index, albumin, calcium, Charlson score, and the duration after transplant at the time of enrollment.

[a]*P* for linear trend.

we adjusted for eGFR in all analyses, and the relationship between FGF23 and poor outcomes was unchanged in prespecified subgroup analyses restricted to patients with intermediate eGFR. These results are consistent with previous reports of similarly robust and "un-confounded" associations between increased FGF23 and progression of CKD, left ventricular hypertrophy, cardiovascular disease events, and mortality, in which FGF23 was also a much more potent predictor of outcomes than serum phosphate or PTH.[4–10] Our results extend those from the only prior reports of mineral metabolism and long-term transplant outcomes in which higher serum phosphate was associated with allograft loss but not consistently associated with mortality.[26,27] Importantly, FGF23 was not measured in either prior study.

A potential limitation of the study is its focus on prevalent transplant recipients recruited at variable durations after transplant. However, the full models were adjusted for transplant vintage, and stratified analyses confirmed that the results were robust to any differences. Furthermore, because increased FGF23 was associated with mortality and allograft loss, any survival bias introduced by recruiting prevalent patients would have been in the direction of preferentially excluding patients with higher FGF23 levels who had already died or reinitiated dialysis, which would have biased the analysis toward the null hypothesis of no association. Studying a prevalent cohort also increased the relevance of the results for clinicians who manage panels of transplant recipients with variable durations of post-transplant time. Additional limitations include the homogenous population of whites, which prevented us from examining FGF23 and outcomes in minority populations; few patients with diabetes or hypertension as the primary cause of renal failure, which may limit generalizability to other transplant populations; the availability of serum rather than plasma for assay of FGF23, which may have led to reporting lower levels; the lack of remaining sample to measure 1,25-dihydroxyvitamin D levels; the lack of stored urine that precluded measurement of phosphate excretion or proteinuria, which is associated with increased FGF23[23] and with allograft loss[28]; and the single time point at which we could measure FGF23 and other analytes. The latter is a limitation of all previous outcomes studies of FGF23 and should be addressed in the future by repeated measures analyses of FGF23 in CKD, dialysis, and transplant populations.

We can speculate on possible mechanisms to explain our results. Deficiencies in the vitamin D axis are associated

with altered immune regulation.[29] Small human and animal studies suggest that 1,25-dihydroxyvitamin D supplementation may have beneficial effects on chronic allograft nephropathy,[30–32] suggesting that FGF23-mediated suppression of 1,25-dihydroxyvitamin D is one possible mechanism through which high FGF23 levels could contribute to allograft loss. In terms of mortality, persistently high FGF23 levels in transplant recipients could reflect the antecedent burden of vascular calcification in the pretransplant period and thereby predict risk of subsequent mortality. FGF23 could also be acting as a sensitive marker of the cardiovascular toxicity of phosphorus accumulation,[33] but this seems less likely in the transplant setting, in which there is overt hypophosphatemia early and persistently lower serum phosphate over the long term than in CKD patients with comparable eGFR.[17] Indeed, the relatively low phosphate and FGF23 levels we observed despite reduced eGFR could suggest total body phosphate depletion. Perhaps FGF23 contributes to phosphate depletion in transplant recipients, which in concert with high PTH and chronic steroid use might aggravate skeletal demineralization and contribute directly to fractures, which, in turn, could increase risk of mortality. However, although transplant recipients have a higher relative risk of fracture compared with dialysis patients,[34] the low absolute risk renders fracture an unlikely explanation for our results. Finally, if chronically increased FGF23 levels can directly stimulate FGFRs in the kidneys and heart independent of klotho,[35] FGF23 could mimic the known effects of FGF2 to induce glomeruloscerosis and cardiac hypertrophy and thereby contribute directly to chronic allograft nephropathy and death.[5,36,37] The consistent finding of increased risk of mortality, cardiovascular disease, and CKD progression among patients with increased FGF23 levels across the diverse clinical settings of dialysis, predialysis, non-CKD, and now, kidney transplantation supports the possibility of FGF23 toxicity. If proven, monoclonal anti-FGF23 antibodies could emerge as a novel therapy for certain kidney transplant recipients.[38]

CONCISE METHODS

Study Population

The Department of Transplantation and Surgery at Semmelweis University in Budapest is the largest kidney transplant center in Hungary. It performs approximately 150 to 170 transplants per year, and the vast majority of recipients continue to receive longitudinal care at the center with minimal loss to follow-up. All stable adult outpatient renal allograft recipients who were followed at the Semmelweis transplant center as of December 31, 2006 ($n = 1214$) were evaluated for inclusion in a prospective cohort study of risk factors for adverse, long-term clinical outcomes among prevalent kidney transplant recipients. Patients were excluded if they were hospitalized or had an episode of acute rejection within the preceding 4 weeks, underwent transplantation in the previous 3 months, or were experiencing an acute infection at the time of recruitment. Two hundred five patients (17%) refused to participate, 16 (1%) satisfied exclusion criteria, and 9 enrollees had inadequate serum samples for complete laboratory testing, which yielded 984 patients for this study. During the 3 years of prospective observation, there was 100% retention and complete follow-up data in all participants. The study protocol was approved by the Institutional Review Board of the Semmelweis University, and all patients provided written informed consent.

Baseline assessments were conducted at enrollment between February and August 2007, during which the following demographic, anthropometric, and past medical data were collected: age, gender, height, weight, body mass index, BP, medical history, medications, primary etiology of CKD, previous time spent on dialysis, and the modified Charlson Comorbidity Index, which has been validated in transplant populations.[39] Transplant data included transplant "vintage," defined as the duration after transplant at the time of enrollment; donor characteristics, including age, gender, deceased or living donor, and cold ischemia time; immunologic factors, including HLA-matching status and titer of panel reactive antibodies; immune suppression regimen; and history of acute rejection or delayed graft function, defined as the need for hemodialysis during the first week after transplantation. During the study, standard maintenance immunosuppressive therapy at Semmelweis consisted of prednisolone plus cyclosporine A or tacrolimus and mycophenolate-mofetil, azathioprine, or sirolimus.

At the baseline assessment, fasting laboratory testing was performed using specimens that were collected in the morning after an overnight fast. Routine laboratory data that were measured for standard clinical practice were measured immediately, and additional aliquots of fasting serum were stored frozen at –70°C for future assay of FGF23. Baseline eGFR was calculated using the four-variable equation derived from the Modification of Diet in Renal Disease Study.[40] Intact PTH was measured by the Elecsys PTH STAT Assay (Elecsys System; Roche, Mannheim, Germany; reference range: 15 to 65 pg/ml), and 25-hydroxyvitamin D was measured with a chemiluminescence immunoassay (DiaSorin, Stillwater, MN; normal range: >75 nmol/L).

Exposures

The primary exposure was the circulating concentration of FGF23, which was measured in duplicate after a single thaw of stored baseline specimens using a second-generation C-terminal ELISA (Immutopics, San Clemente, CA). The inter- and intra-assay coefficients of variation were 8.3 and 5.0%, respectively. FGF23 assays are highly correlated in CKD, and a recent report of peritoneal dialysis patients showed that virtually all circulating FGF23 was intact, biologically active, and accurately measured by C-terminal or intact assays.[24]

The secondary exposures were PTH and serum phosphate. We analyzed risk of adverse outcomes according to FGF23, PTH, and serum phosphate on a continuous scale after using natural log transformation of FGF23 and PTH to achieve normality. To assess for nonlinear effects and

Table 6. Hazard ratios of the composite outcome of death or allograft loss according to serum phosphate (per 1 SD increase) and ascending tertiles of phosphate

	Phosphate		Phosphate Tertiles			
	per 1 SD	*P*	1	2	3	*P*[a]
All patients, $n = 984$						
crude	1.26	<0.001	Reference	1.06	2.87	<0.001
	1.19, 1.33			0.69, 1.64	1.99, 4.13	
eGFR	1.25	<0.001	Reference	0.83	1.36	0.06
	1.15, 1.36			0.54, 1.29	0.92, 2.01	
eGFR, age, gender	1.28	<0.001	Reference	0.86	1.53	0.02
	1.18, 1.39			0.56, 1.33	1.02, 2.28	
full model	1.28	<0.001	Reference	0.83	1.18	0.32
	1.14, 1.43			0.54, 1.29	0.78, 1.79	
full model + logPTH	1.26	<0.001	Reference	0.84	1.21	0.27
	1.12, 1.42			0.54, 1.31	0.80, 1.84	
full model + logFGF23	1.23	0.002	Reference	0.84	1.09	0.57
	1.08, 1.40			0.54, 1.31	0.71, 1.66	
Restricted to patients with eGFR 30 to 90 ml/min per 1.73 m^2, $n = 765$						
crude	0.95	0.73	Reference	0.97	1.18	0.54
	0.74, 1.24			0.60, 1.57	0.72, 1.93	
eGFR	0.83	0.23	Reference	0.86	0.94	0.79
	0.61, 1.13			0.53, 1.40	0.57, 1.55	
eGFR, age, gender	0.86	0.35	Reference	0.88	1.03	0.95
	0.63, 1.18			0.54, 1.43	0.61, 1.72	
full model	0.84	0.27	Reference	0.86	0.96	0.85
	0.61, 1.15			0.52, 1.41	0.57, 1.61	
full model + logPTH	0.86	0.33	Reference	0.87	1.02	0.99
	0.63, 1.17			0.53, 1.42	0.60, 1.71	
full model + logFGF23	0.80	0.15	Reference	0.85	0.88	0.62
crude	0.58, 1.08			0.52, 1.40	0.52, 1.48	

Full model is adjusted for eGFR, age, gender, systolic BP, body mass index, albumin, calcium, Charlson score, and the duration after transplant at the time of enrollment.
[a]*P* for linear trend.

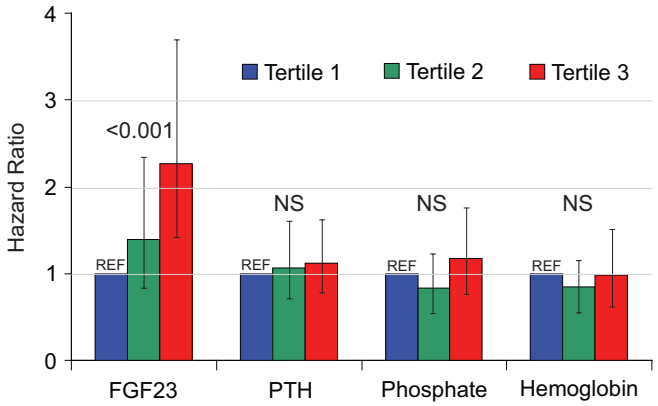

Figure 2. Ascending tertiles of FGF23 but not PTH, phosphate, or hemoglobin independently associate with greater risk of the composite outcome of all-cause mortality and allograft loss. Hazard ratios for the composite outcome of all-cause mortality and allograft loss according to tertiles of FGF23, PTH, phosphate, and hemoglobin derived from the respective full multivariable models. For each exposure, the lowest tertile served as the referent group (REF). Ascending tertiles of FGF23 were independently associated with increased risk of the composite outcome ($P < 0.001$), but the relationships between the other analytes and outcomes were all nonsignificant (NS).

to aid clinical interpretation, we also examined each exposure in tertiles according to its distribution in the overall study population.

Outcomes

Kidney transplant recipients underwent prospective follow-up until they died, returned to dialysis, or the observation period ended. The primary outcome was the composite of all-cause mortality or allograft loss necessitating a return to dialysis. The secondary outcomes were each component of the composite outcome analyzed separately. Death and re-initiation of maintenance dialysis were ascertained from the hospital's electronic medical records and validated by cross-referencing national registries.

Statistical Analysis

We used descriptive statistics to present baseline characteristics at the time of entry into the cohort for the overall population and compared differences across tertiles of FGF23 using one-way ANOVA, Kruskal-Wallis test, and χ^2 tests as appropriate. We used Spearman correlation to assess univariate relationships between exposures, eGFR, and transplant vintage.

We used several complementary analytic strategies to examine risk of mortality and allograft loss according to FGF23. First, we examined the association between baseline FGF23 and the composite outcome of death or allograft loss censoring only for the end

of the observation period because no patients were lost to follow-up. Next, we separately analyzed the association of the primary exposures with all-cause mortality and death-censored allograft loss. Because death precluded the occurrence of allograft loss and allograft loss with return to dialysis precluded the occurrence of death, we used separate competing risk analyses of the specific outcomes.[41] We used cumulative incidence plots to present univariate analyses of FGF23 tertiles and outcomes and tested for statistically significant trends across tertiles using log-rank tests.

After confirming the proportionality assumption, we used Cox proportional hazards models to adjust for confounding in the analysis of the primary composite outcome and used competing risks regression to adjust for confounding in analyses of the individual endpoints. The multivariable modeling strategy involved several layers, was prespecified, and was consistent across all exposures. Because we expected both FGF23 levels and clinical outcomes to be most strongly confounded by renal function, we first adjusted for eGFR in all models. Next, we used hierarchical models to adjust for age and gender, and then we adjusted for these factors plus systolic BP, body mass index, albumin, calcium, Charlson score, and transplant vintage in the full models. To further assess the impact of transplant vintage, we also examined full multivariable models stratified by tertiles of transplant vintage. We further adjusted the full models for serum phosphate and then natural log-transformed PTH to test whether the effect of FGF23 was independent of other markers of disordered phosphorus metabolism. Because a significant proportion of transplant recipients manifest persistently low serum phosphate levels in contrast to CKD patients, we examined the association between FGF23 and outcomes across tertiles of phosphate. We also adjusted for donor type, history of rejection or delayed graft function, and immune suppressive therapy, given a report that suggested that steroids are associated with increased FGF23.[42] Fewer events limited the number of covariates that could be included in the separate competing risk analyses of death and death-censored allograft loss. Therefore, we adjusted for eGFR and then eGFR, age, and gender in these secondary analyses.

Vitamin D levels were not associated with the outcomes and were excluded from the multivariable modeling. However, because therapy with 1,25-dihydroxyvitamin D increases and phosphate binders decrease FGF23 levels,[20,43] and each has been independently associated with decreased mortality,[44,45] we further adjusted the main models for therapy with these agents. Finally, given previous reports of higher FGF23 levels in association with diabetes and inflammation,[46] we reanalyzed the main models after adjusting for diabetes and for C-reactive protein levels and white blood cell count as measures of inflammation.

Sensitivity Analyses

In a prespecified analysis aimed at further addressing whether the effect of FGF23 is independent of eGFR, we repeated the main analyses after excluding patients with the highest (>90 ml/min per 1.73 m^2) and lowest (<30 ml/min per 1.73 m^2) eGFR at the outset of the observation period when FGF23 was measured. This excluded patients who were at the low and high extremes of risk of death or allograft loss and enabled us to focus on the subgroup of patients whose future risk of events would be least clear clinically. In the analyses of these 765 patients, we used the identical hierarchical modeling strategy and covariates as described above, which facilitated comparison with the analyses of the overall study population.

Like FGF23, levels of PTH and serum phosphate increase as renal function deteriorates, and each has been independently associated with mortality in previous studies.[13,14] To further examine whether any relationship between FGF23 excess and adverse outcomes is specific to FGF23 or represents a global relationship with markers of disordered phosphorus metabolism, we repeated the analysis strategy described above after substituting PTH and serum phosphate for FGF23 as the primary exposure. As an additional, nonmineral metabolism "control" for the degree of renal dysfunction, we also performed a parallel analysis of hemoglobin levels as the primary exposure. All analyses were performed using Intercooled Stata 11 (College Station, TX). $P < 0.05$ was considered statistically significant.

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DISCLOSURES

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See related editorial, "New Insights to Fibroblast Growth Factor 23 in Kidney Transplant," on pages 799–801.