Impact of etelcalcetide on fibroblast growth factor-23 and calciprotein particles in patients with secondary hyperparathyroidism undergoing hemodialysis

Yusaku Hashimoto, MD[1]; Sawako Kato, MD, PhD[1]*; Makoto Kuro-o MD, PhD[2]; Yutaka Miura, PhD[2]; Yuya Itano, MD[1]; Masahiko Ando, MD, PhD[3]; Yachiyo Kuwatsuka, MD, PhD[3]; and Shoichi Maruyama, MD, PhD[1]

1 Department of Nephrology, Nagoya University Graduate School of Medicine, Aichi, Japan

2 Division of Anti-aging Medicine, Center for Molecular Medicine, Jichi Medical University, Tochigi, Japan

3 Department of Advanced Medicine, Nagoya University Hospital, Aichi, Japan

Corresponding author's information: Sawako Kato, M.D., Ph.D.

Department of Nephrology, Nagoya University Graduate School of Medicine

65 Tsuruma-cho, Showa-ku, Nagoya, Aichi 464-8550, Japan

Tel: +81-52-744-2192; Fax: +81-52-744-2209

Email: kato07@med.nagoya-u.ac.jp

A short running title: Impact of Etelcalcetide on FGF23 and CPPs

Summary at a Glance

This *post hoc* analysis showed that etelcalcetide may be useful through suppression of fibroblast growth factor-23 (FGF23) levels in the treatment of secondary hyperparathyroidism. When correcting hypocalcemia, loading oral calcium preparations could be more advantageous than active vitamin D for the suppression of both FGF23 and calciprotein particles.

Abstract

Background: Recently, we demonstrated the efficacy of etelcalcetide in the control of secondary hyperparathyroidism (SHPT). This *post hoc* analysis aimed to evaluate changes in fibroblast growth factor-23 (FGF23) and calciprotein particles (CPPs) after treatment with calcimimetics.

Methods: The DUET trial was a 12-week multicenter, open-label, parallel-group, randomized (1:1:1) study with patients treated with etelcalcetide plus active vitamin D (E+D group; n = 41), etelcalcetide plus oral calcium (E+Ca group; n = 41), or control (C group; n = 42) under maintenance hemodialysis. Serum levels of FGF23 and CPPs were measured at baseline, and 6 and 12 weeks after the start.

Results: In the linear mixed model, serum levels of FGF23 in etelcalcetide users were significantly lower than those in non-users at week 6 ($p < 0.001$) and week 12 ($p < 0.001$). When compared the difference between the E+Ca group and the E+D group, serum levels of FGF23 in the E+Ca group were significantly lower than those in the E+D group at week 12 ($p = 0.017$). There were no significant differences in the serum levels of CPPs between etelcalcetide users and non-users at week 6 ($p = 0.10$) and week 12 ($p = 0.18$), while CPPs in the E+Ca group were significantly lower than those in the E+D group ($p < 0.001$) at week 12.

Conclusion: Etelcalcetide may be useful through suppression of FGF23 levels among hemodialysis patients with SHPT. When correcting hypocalcemia, loading oral calcium preparations could be more advantageous than active vitamin D for the suppression of both FGF23 and CPPs.

Key words: calciprotein particles, etelcalcetide, fibroblast growth factor-23, hypocalcemia, secondary hyperparathyroidism

1 INTRODUCTION

As the prevalence of cardiovascular disease (CVD) is significantly high in patients with chronic kidney disease (CKD), reducing the risk of CVD is a priority for CKD management [1]. Vascular calcification (VC) is one of initial signs of CVD in patients with CKD, often inducing reduced arterial compliance and impaired cardiovascular function [2]. To prevent VC, management of CKD-mineral bone disease (MBD), including controlling the optimal levels of phosphate, calcium, and parathyroid hormone (PTH) is broadly recommended [3]. Calciprotein particles (CPPs), colloidal nanoparticles of calcium-phosphate precipitants attached to a serum protein fetuin-A, are regarded as causal agents of VC [4]. It was reported that CPPs reflected extraosseous calcification stress in CKD patients including under hemodialysis (HD) [5] and associated with aortic stiffness in pre-dialysis patients [6]. Moreover, higher CPPs levels were associated with CVD events in the prospective observational study enrolled maintenance HD patients [7]. Fibroblast growth factor-23 (FGF23) is a bone-derived phosphaturic hormone that acts with its cofactor alpha-klotho [8] and is thought to cause cardiovascular complications [9]. Indeed, there are many reports that state that FGF23 is a predictor of CVD and CVD mortality in patients undergoing dialysis, as well as in patients pre-dialysis

[10, 11].

Etelcalcetide, a calcimimetic agent used to treat secondary hyperparathyroidism (SHPT), is one of the most powerful calcimimetics to lower intact PTH (iPTH) levels, although it is more susceptible to hypocalcemia than other calcimimetics [12]. Recently, we conducted a randomized controlled study named “DUET study” in patients undergoing maintenance HD, which showed the predominant efficacy of etelcalcetide in controlling SHPT [13]. We also confirmed the advantage of active vitamin D in correcting hypocalcemia and superiority of oral calcium preparation for suppression of hyperphosphatemia, when evaluating which it was better to load vitamin D or oral calcium preparation for correction of hypocalcemia [13]. Some studies have reported that FGF23 levels decrease in parallel with iPTH levels during SHPT treatment treated with vitamin D and calcimimetics [14, 15]. To date, few reports have focused on changes in FGF23 levels by comparing correction drugs for hypocalcemia [16], and no study has evaluated CPP levels during treatment with etelcalcetide.

In this secondary analysis of the DUET study, we aimed to evaluate the impact of eletcalcetide on serum levels of FGF23 and CPPs, and focus on how these biomarkers changed depending on which drugs were selected, active

vitamin D or oral calcium preparations, for the correction of consequent hypocalcemia. Moreover, we examined the correlations between changes in FGF-23, CPPs, and other CKD-MBD-related markers. These results could contribute to the development of new treatment strategies for SHPT to reduce CVD risk in patients undergoing HD.

2 MATERIALS AND METHODS

2.1 Study populations

The DUET study was a 12-week, multicenter, open-label, randomized (1:1:1), parallel-group study [13], which assigned 124 patients with mild SHPT undergoing maintenance HD to three groups of etelcalcetide plus active vitamin D (group E+D), etelcalcetide plus oral calcium preparation (group E+Ca), and control (group C). In the event of hypocalcemia induced by etelcalcetide, we administered active vitamin D and oral calcium preparations in addition to the original medications to patients allocated to groups E+D and E+Ca, respectively. This trial was approved by the Nagoya University Clinical Research Review Board (CRB4180004) and was consistent with the 1964 Helsinki Declaration. The detailed rationale and research design of the DUET study have already been released [17]. This study was a post-hoc analysis of the DUET study. All patients provided written consent for participation in the DUET study including this sub analysis.

2.2 Biomarkers and biochemical data

We preserved the blood samples collected at baseline, and 6 weeks and 12

weeks after the start of the trial. Blood FGF23 levels were measured in the laboratory of SRL Inc., Aichi, Japan. The method was previously reported as "automated chemiluminescence immunoassay" [18]. Briefly, samples were mixed in a disposable cuvette with biotinylated anti-FGF23 N-terminal monoclonal antibody, alkaline phosphatase-labeled anti-FGF23 C-terminal monoclonal antibody, and streptavidin magnetic particles as the solid phase. After washing, an alkaline phosphatase detection substate was added, and FGF23 was measured using a photon counter.

Blood CPP levels were measured at the Division of Anti-Aging Medicine, Center for Molecular Medicine, Jichi Medical University. Measuring methods of blood CPP levels used was previously reported as "gel filtration method" [19]. I In brief, serum or plasma samples were inoculated with OsteoSense® (PerkinElmer, Chicago, USA), a bisphosphonate labeled with an infrared fluorescent dye that bound to calcium phosphate crystals, incubated for 60 min at 25 °C, and then applied to a gel filtration spin column to remove unbound OsteoSense®. The fluorescence intensity of the CPP-containing flow-through was quantified using an infrared fluorescence scanner and designated as the total CPP level. CPPs are colloidal particles composed of solid-phase calcium phosphate (CaPi) and

serum protein fetuin-A. Secondary CPPs are defined as CPPs containing crystalline CaPi. Primary CPPs are defined as CPPs that do not contain crystalline CaPi but contain non-crystalline (amorphous) CaPi. Secondary CPPs, but not primary CPPs, can induce calcification when applied to cultured vascular smooth muscle cells [20]. The gel filtration method uses a fluorescent probe that binds to crystalline CaPi, but not to amorphous CaPi. Thus, the gel filtration method primarily measures secondary CPPs. Other biochemical data including phosphorus (P), calcium (Ca), and iPTH were collected from the DUET study.

2.3 Statistical analysis

Skewed data (FGF23 and CPP) were transformed to natural logarithm to achieve a normal distribution before statistical analysis. The changes in $\log_e$ FGF23 and $\log_e$ CPP were estimated in a linear mixed models with each treatment group, time point, and interaction of the treatment group and time point as fixed effects. We compared these changes between treatment groups using a linear mixed model and the Tukey-Kramer method to correct for multiplicity. In the comparison, the etelcalcetide users (groups E+D and E+Ca) and the control group (non-etelcalcetide users) were compared; only etelcalcetide users were compared in

the group divided based on the correction method for hypocalcemia (group E+D vs. group E+Ca).

We further examined the correlation between the decrease in FGF23, CPP, and other parameters related to bone mineral metabolism (iPTH, Ca, P, and Ca-P product) using Spearman's rank correlation coefficient. Serum Ca levels were corrected for serum albumin levels. The percent change was calculated from baseline to 6 weeks at the start of trial = [(6 weeks - baseline)/ baseline] × 100 (%) and from 6 weeks to 12 weeks = [(12 weeks - baseline)/ baseline] × 100 (%). First, we calculated the correlations of the parameters in all patients, regardless of the intervention group. Next, we examined this correlation in patients divided by the treatment groups. All statistical analyses were performed using Stata version 16.0 MP (Stata Corp, www.stata.com) and SAS software (SAS Institute Inc. Cary, NC, USA). Statistical significance was set at $p < 0.05$.

3 RESULTS

3.1 Study population

Characteristics of the patients enrolled to the DUET study at baseline was described in the main paper [13] and supplemented in Table S1.

3.2 Fibroblast growth factor-23 (FGF23) over time

The median (range) of FGF23 were 5800 pg/mL (78-59000) at baseline, 3650 pg/mL (45-41700) at 6 weeks, and (12-71200) at 12 weeks after the start of the trial in the whole enrolled patients. We calculated estimated FGF23 levels using a linear mixed model, and plotted ln FGF23 of the etelcalcetide administration group and group C (Figure 1A) and the E+D and E+Ca groups (Figure 1B). ln FGF23 levels in patients treated with etelcalcetide were significantly lower than those in the control patients at 6 weeks (etelcalcetide vs control, 7.57 vs 8.59 pg/mL; $p < 0.001$) and 12 weeks (7.59 vs 8.62 pg/mL, $p < 0.001$). FGF23 levels in the E+Ca group were significantly lower than those in the E+D group at 12 weeks after the start of the trial (E+Ca group vs. E+D group, 6.87 vs 8.24 pg/mL; $p < 0.0001$), although there was no significant difference at 6 weeks.

3.3 Calciprotein particles (CPPs) over time

The median (range) of CPPs were 46170 AU (5474-349113) at baseline, 18206.5 AU (5026-28036) at 6 weeks, and 15829.5 AU (4116-146755) at 12 weeks after the start of the trial in the whole enrolled patients. We calculated the estimated CPPs using the same statistical method as for FGF23 and plotted ln CPPs (Figure 2). There was no significant difference in ln CPPs between the etelcalcetide group and group C at 6 or 12 weeks from the start of the trial (Figure 2A). CPP levels were decreased over time in group C without treatment with etelcalcetide. However, when compared among etelcalcetide-administered groups, ln CPPs were significantly lower in the E+Ca group compared to the E+D group (E+Ca group vs. E+D group, 9.44 vs 10.18 AU; $p = 0.0047$) at 12 weeks although there was no significant difference at 6 weeks (Figure 2B).

3.4 Positive correlations of the decrease in bone mineral-markers with decreased FGF23 and CPPs in all patients who enrolled in this trial

Table 1 shows the Spearman correlation coefficients between changes in FGF23 and the representative bone mineral markers, and CPPs and these markers from baseline to 6 weeks from the start of the trial (upper panel) and from baseline to

12 weeks (lower panel). The decrease in FGF23 levels positively correlated with decreases in corrected serum Ca ($\rho = 0.42$, $p < 0.001$), P ($\rho = 0.48$, $p < 0.001$), and Ca-P product ($\rho = 0.66$, $p < 0.001$) from baseline to 6 weeks, and in Ca ($\rho = 0.47$, $p < 0.001$), P ($\rho = 0.63$, $p < 0.001$), and Ca-P product ($\rho = 0.73$, $p < 0.001$) from baseline to 12 weeks, but there was no correlation with decreases in iPTH at any time point.

The decrease in CPPs was positively correlated with the decrease in phosphate from baseline to Week 6 ($\rho = 0.47$, $p < 0.001$) and from baseline to Week 12 ($\rho = 0.56$, $p < 0.001$), but not with Ca and iPTH. The decreases in FGF23 and CPP were weakly correlated from baseline to Week 6 ($\rho = 0.29$, $p = 0.001$) and from baseline to Week 12 ($\rho = 0.56$, $p = 0.001$).

3.5 Correlations of changes in Ca-P product with changes in FGF23 and CPPs in each patient group due to treatment of SHPT

We evaluated whether treatment with etelcalcetide caused a difference in these correlations. Figure 3 shows the changes in FGF23 and the Ca-P product. Changes in FGF23 levels were correlated with changes in the Ca-P product in the etelcalcetide group (baseline to week 6: $\rho = 0.58$, $p < 0.001$; baseline to week

12: ρ = 0.75, p < 0.001) and in the C group (baseline to week 6: ρ = 0.59, p = 0.001; week 6 to week 12: ρ = 0.71, p < 0.001).

Figure 4 shows the changes in CPPs and the Ca-P product. Similarly, changes in CPPs were correlated with changes in the Ca-P product in the etelcalcetide group (baseline to week 6: ρ = 0.41, p < 0.001; baseline to week 12: ρ = 0.56, p < 0.001) and in the C group (baseline to week 6: ρ = 0.56, p < 0.001; baseline to week 12: ρ = 0.61, p < 0.001).

4 DISCUSSION

In this post-hoc analysis, we evaluated serum FGF23 and CPP levels in patients undergoing maintenance HD who were treated for mild to moderate SHPT [mean baseline iPTH; 296 pg/mL (range 96-717 pg/mL, median 271 pg/mL)] with or without etelcalcetide. We verified that FGF23 levels were significantly lower in patients using etelcalcetide than in those receiving conventional treatment without calcimimetics. Although CPP levels were lower in patients using etelcalcetide than in non-users, the difference was not statistically significant. We also compared the serum FGF23 and CPP levels in patients receiving calcium preparation with those in patients receiving active vitamin D when correcting for hypocalcemia induced by etelcalcetide. We found that both FGF23 and CPP levels were significantly lower in patients with calcium preparations than in those with active vitamin D. This is the first study to demonstrate that intentionally loading oral calcium preparation rather than loading active vitamin D can control SHPT without elevating FGF23 and CPP levels for correction of hypocalcemia induced by etelcalcetide.

Several previous studies have reported that treatment with calcimimetics lowered serum FGF23 levels [12, 14, 21-23]. One secondary analysis indicated that

loading concomitant vitamin D partially attenuated the FGF23-lowering effect of etelcalcetide in patients with severe SHPT, while loading calcium preparation had no effect [16]. A comparative trial between cinacalcet and active vitamin D revealed that changes in FGF23 levels were correlated with changes in Ca-P products that resulted in decreased and increased FGF23 levels in the cinacalcet arm and active vitamin D arm, respectively [14]. The FGF23-lowering effect of etelcalcetide demonstrated in our study was consistent with the results of previous studies [12, 14, 21-23]. We also confirmed a significant decrease in FGF23 levels in patients with oral calcium preparation and an increase in patients with vitamin D administration. This result was compatible with the previous correlation between changes in FGF23 and Ca-P products. Since, FGF23 should be a predictor of mortality in dialysis patients, our results suggest that calcimimetics including etelcalcetide may be first recommended to control SHPT, and then calcium preparation may be preferred to avoid an increase in FGF23 for correction of consequent hypocalcemia.

There have been fewer clinical studies evaluating CPP than FGF23 in patients undergoing HD [24, 25], especially few interventional studies [26, 27] and none with cases treated by calcimimetics. A prospective observational study showed

an increase in CPPs in proportion to an increase in iPTH and calcium levels after discontinuation of cinacalcet [25]. Two interventional studies reported that non-calcium-containing P binders were associated with lower CPP than calcium carbonate [26, 27]. Interestingly, sevelamer was associated with reduced inflammation and improved vascular function in correlation with a reduction in CPP [27], and the changes in CPPs were positively correlated with those in LDL cholesterol under use of lanthanum carbonate [26]. The etelcalcetide significantly prolonged CPP maturation time (T50) compared with vitamin D [28], which means etelcalcetide has more advantageous suppressing calcification propensity than vitamin D. In our study, etelcalcetide failed to significantly reduce CPP compared to no use of etelcalcetide. In the correction of hypocalcemia, loading calcium preparation significantly reduced CPP compared to loading active vitamin D. Our results suggest that calcium preparation may have an advantage in protecting vascular calcification for correction of consequent hypocalcemia.

The mechanism by which calcimimetics affect FGF23 and CPPs is not fully understood. Osteocytes/osteoblasts are the major source of FGF23 production in response to phosphate load, but it was not known how these cells can sense phosphate load. Akiyama et al [4] reported that CPPs are acting as the

phosphate stimulus to these cells. Therefore, a higher level of CPP is correlated with a higher FGF23. Treatment with etelcalcetide reduces serum calcium, phosphate, and CPP levels, which may be a cause of FGF23 reduction following etelcalcetide treatment. And calcimimetics may act to suppress FGF23 directly on calcium-sensing receptors in bone cells and/or indirectly via a decrease in Ca-P products [4, 8]. A decrease in FGF23 levels strongly correlated with decrease in Ca-P product during treatment of SHPT using calcimimetics and placebo, which supported the indirect pathway of change in FGF23 at least [16]. As a report supporting the direct effect of calcimimetics, a comparative interventional study of cinacalcet and vitamin D showed a significant decrease in FGF23 in cinacalcet arm, although the relationship between FGF23 and Ca-P products in cinacalcet arm was weaker than that in the vitamin D arm [14]. Our results supported the indirect pathway due to the correlation of FGF23 with the Ca-P product, but failed to support the direct pathway since there was no difference in FGF23 changes between patients using etelcalcetide and non-users. Although there have been reports on an association between PTH and FGF23 [29, 30], PTH has been speculated to have no impact on FGF23 changes under calcimimetic use [14-16], which is compatible with our results. CPP has been reported to increase in

correlation with serum P levels [19], but no study has evaluated the change in CPP under treatment with calcimimetics. In a previous report which studied patients after cessation of calcimimetics, changes in total CPP were associated with changes in PTH, but not P, although only changes in CPPs containing crystalline Ca-P transformed from primary CPPs, called secondary CPP, were associated with changes in P [25]. In our study, which was the first to measure CPP during SHPT treatment with calcimimetics, we demonstrated that decrease in CPP was positively correlated with decrease in P, but not Ca or iPTH, which supports the importance of controlling P to suppress production of CPP in SHPT.

4.1 Limitations and strengths

One of the limitations is that we only investigated surrogate markers as endpoints, not direct detection of VC or cardiovascular events. Another limitation is that the subjects were only Japanese with mild SHPT, and it should have been more informative if this trial applied to other races or patients with other severities of SHPT. In addition, although the quality of CPP, including T50 and high-density CPP (larger size and high density), has been shown to be clinically more meaningful in pathogenesis than CPP in total quantity [19, 24, 28], our study is the

first to simultaneously measure the effects of etelcalcetide on FGF23 and CPP, and compare the effects of etelcalcetide-induced hypocalcemia medication on these biomarkers, which is the strength of our study.

4.2 Conclusion and future directions

In the treatment of mild SHPT in patients undergoing maintenance HD, selection of etelcalcetide may have an advantage in suppression of FGF23. Moreover, when correcting hypocalcemia induced by etelcalcetide, loading oral calcium preparations could be recommended from the point of suppression of both FGF23 and CPPs. Although future studies are needed, we believe that our study provides useful knowledge for developing better CKD-MBD treatment strategies.

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CONFLICT OF INTEREST STATEMENT

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AUTHORS' CONTRIBUTIONS

YH and SK were contributed to the research idea, the study concept, analysis, and interpretation of data, and were primarily responsible for literature review and writing the paper. MK and YM were responsible for measuring CPP, interpreting the data, and writing the paper. MA compiled the data management system. YK performed the statistical analyses. SM was also responsible for the research idea, the study concept, designing and organizing the study as a principal investigator, and supervising the writing of the paper.

ORCID

Sawako Kato https://orcid.org/0000-0001-7210-1888

REFERENCES

1 Chen TK, Knicely DH, Grams ME. Chronic Kidney Disease Diagnosis and Management: A Review. JAMA. 2019; 322: 1294-304.

2 Reiss AB, Miyawaki N, Moon J, Kasselman LJ, Voloshyna I, D'Avino R, Jr., et al. CKD, arterial calcification, atherosclerosis and bone health: Inter-relationships and controversies. Atherosclerosis. 2018; 278: 49-59.

3 Ketteler M, Block GA, Evenepoel P, Fukagawa M, Herzog CA, McCann L, et al. Executive summary of the 2017 KDIGO Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) Guideline Update: what's changed and why it matters. Kidney Int. 2017; 92: 26-36.

4 Akiyama KI, Miura Y, Hayashi H, Sakata A, Matsumura Y, Kojima M, et al. Calciprotein particles regulate fibroblast growth factor-23 expression in osteoblasts. Kidney Int. 2020; 97: 702-12.

5 Hamano T, Matsui I, Mikami S, Tomida K, Fujii N, Imai E, et al. Fetuin-mineral complex reflects extraosseous calcification stress in CKD. J Am Soc Nephrol. 2010; 21: 1998-2007.

6 Smith ER, Ford ML, Tomlinson LA, Rajkumar C, McMahon LP, Holt SG. Phosphorylated fetuin-A-containing calciprotein particles are associated with

aortic stiffness and a procalcific milieu in patients with pre-dialysis CKD. Nephrol Dial Transplant. 2012; 27: 1957-66.

7 Gatate Y, Nakano S, Mizuno Y, Muramatsu T, Senbonmatsu T, Nishimura S, et al. Mid-term predictive value of calciprotein particles in maintenance hemodialysis patients based on a gel-filtration assay. Atherosclerosis. 2020; 303: 46-52.

8 Kuro OM, Moe OW. FGF23-alphaKlotho as a paradigm for a kidney-bone network. Bone. 2017; 100: 4-18.

9 Kuro OM. Klotho and endocrine fibroblast growth factors: markers of chronic kidney disease progression and cardiovascular complications? Nephrol Dial Transplant. 2019; 34: 15-21.

10 Gao S, Xu J, Zhang S, Jin J. Meta-Analysis of the Association between Fibroblast Growth Factor 23 and Mortality and Cardiovascular Events in Hemodialysis Patients. Blood Purif. 2019; 47 Suppl 1: 24-30.

11 Komaba H, Fuller DS, Taniguchi M, Yamamoto S, Nomura T, Zhao J, et al. Fibroblast Growth Factor 23 and Mortality Among Prevalent Hemodialysis Patients in the Japan Dialysis Outcomes and Practice Patterns Study. Kidney Int Rep. 2020; 5: 1956-64.

12 Block GA, Bushinsky DA, Cheng S, Cunningham J, Dehmel B, Drueke TB, et al. Effect of Etelcalcetide vs Cinacalcet on Serum Parathyroid Hormone in Patients Receiving Hemodialysis With Secondary Hyperparathyroidism: A Randomized Clinical Trial. JAMA. 2017; 317: 156-64.

13 Itano Y, Kato S, Tsuboi M, Kasuga H, Tsuruta Y, Sato F, et al. A Prospective, Randomized Clinical Trial of Etelcalcetide in Patients Receiving Hemodialysis With Secondary Hyperparathyroidism (the DUET Trial). Kidney Int Rep. 2020; 5: 2168-77.

14 Sprague SM, Wetmore JB, Gurevich K, Da Roza G, Buerkert J, Reiner M, et al. Effect of Cinacalcet and Vitamin D Analogs on Fibroblast Growth Factor-23 during the Treatment of Secondary Hyperparathyroidism. Clin J Am Soc Nephrol. 2015; 10: 1021-30.

15 Shigematsu T, Fukagawa M, Yokoyama K, Akiba T, Fujii A, Odani M, et al. Effects of the Intravenous Calcimimetic Etelcalcetide on Bone Turnover and Serum Fibroblast Growth Factor 23: Post Hoc Analysis of an Open-label Study. Clin Ther. 2018; 40: 2099-111.

16 Wolf M, Block GA, Chertow GM, Cooper K, Fouqueray B, Moe SM, et al. Effects of etelcalcetide on fibroblast growth factor 23 in patients with secondary

hyperparathyroidism receiving hemodialysis. Clin Kidney J. 2020; 13: 75-84.

17 Kato S, Tsuboi M, Ando M, Itano Y, Maruyama S. Rationale and study design of a randomized controlled trial for development of a treatment strategy for chronic kidney disease–mineral and bone disorder by multilateral mechanism of etelcalcetide hydrochloride (the DUET study). Renal Replacement Therapy. 2019; 5: 39.

18 Shimizu Y, Fukumoto S, Fujita T. Evaluation of a new automated chemiluminescence immunoassay for FGF23. J Bone Miner Metab. 2012; 30: 217-21.

19 Miura Y, Iwazu Y, Shiizaki K, Akimoto T, Kotani K, Kurabayashi M, et al. Identification and quantification of plasma calciprotein particles with distinct physical properties in patients with chronic kidney disease. Sci Rep. 2018; 8: 1256.

20 Aghagolzadeh P, Bachtler M, Bijarnia R, Jackson C, Smith ER, Odermatt A, et al. Calcification of vascular smooth muscle cells is induced by secondary calciprotein particles and enhanced by tumor necrosis factor-alpha. Atherosclerosis. 2016; 251: 404-14.

21 Block GA, Bushinsky DA, Cunningham J, Drueke TB, Ketteler M,

Kewalramani R, et al. Effect of Etelcalcetide vs Placebo on Serum Parathyroid Hormone in Patients Receiving Hemodialysis With Secondary Hyperparathyroidism: Two Randomized Clinical Trials. JAMA. 2017; 317: 146-55.

22 Hryszko T, Brzosko S, Rydzewska-Rosolowska A, Koc-Zorawska E, Mysliwiec M. Cinacalcet lowers FGF-23 level together with bone metabolism in hemodialyzed patients with secondary hyperparathyroidism. Int Urol Nephrol. 2012; 44: 1479-86.

23 Moe SM, Chertow GM, Parfrey PS, Kubo Y, Block GA, Correa-Rotter R, et al. Cinacalcet, Fibroblast Growth Factor-23, and Cardiovascular Disease in Hemodialysis: The Evaluation of Cinacalcet HCl Therapy to Lower Cardiovascular Events (EVOLVE) Trial. Circulation. 2015; 132: 27-39.

24 Pasch A, Block GA, Bachtler M, Smith ER, Jahnen-Dechent W, Arampatzis S, et al. Blood Calcification Propensity, Cardiovascular Events, and Survival in Patients Receiving Hemodialysis in the EVOLVE Trial. Clin J Am Soc Nephrol. 2017; 12: 315-22.

25 Ruderman I, Smith ER, Toussaint ND, Hewitson TD, Holt SG. Longitudinal changes in bone and mineral metabolism after cessation of cinacalcet in dialysis patients with secondary hyperparathyroidism. BMC Nephrol.

2018; 19: 113.

26 Nakamura K, Nagata Y, Hiroyoshi T, Isoyama N, Fujikawa K, Miura Y, et al. The effect of lanthanum carbonate on calciprotein particles in hemodialysis patients. Clin Exp Nephrol. 2020; 24: 323-29.

27 Smith ER, Pan FFM, Hewitson TD, Toussaint ND, Holt SG. Effect of Sevelamer on Calciprotein Particles in Hemodialysis Patients: The Sevelamer Versus Calcium to Reduce Fetuin-A-Containing Calciprotein Particles in Dialysis (SCaRF) Randomized Controlled Trial. Kidney Int Rep. 2020; 5: 1432-47.

28 Shoji T, Nakatani S, Kabata D, Mori K, Shintani A, Yoshida H, et al. Comparative Effects of Etelcalcetide and Maxacalcitol on Serum Calcification Propensity in Secondary Hyperparathyroidism: A Randomized Clinical Trial. Clin J Am Soc Nephrol. 2021; 16: 599-612.

29 Sato T, Tominaga Y, Ueki T, Goto N, Matsuoka S, Katayama A, et al. Total parathyroidectomy reduces elevated circulating fibroblast growth factor 23 in advanced secondary hyperparathyroidism. Am J Kidney Dis. 2004; 44: 481-7.

30 Wesseling-Perry K, Harkins GC, Wang HJ, Elashoff R, Gales B, Horwitz MJ, et al. The calcemic response to continuous parathyroid hormone (PTH)(1-34) infusion in end-stage kidney disease varies according to bone turnover: a

potential role for PTH(7-84). J Clin Endocrinol Metab. 2010; 95: 2772-80.

SUPPORTING INFORMATION LEGEND

Additional supporting information may be found online in the Supporting Information section at the end of this article.

Table S1

Values of mineral metabolism index and use of oral calcium preparation and vitamin D in three groups and three periods

Figure Legends

FIGURE 1

Time course of log_e fibroblast growth factor-23 (ln FGF23) changes by treatment. Line plot showing ln FGF23 (points) that was estimated in a linear mixed model after transformed to natural logarithm and SD (error bars). Asterisks connote statistical significance of $p < 0.05$.

FIGURE 2

Time course of l log_e calciprotein particles (ln CPPs) changes by treatment. Line plot showing ln CPPs (points) that was estimated in a linear mixed model after transformed to natural logarithm and SD (error bars). An asterisk connote statistical significance of $p < 0.05$.

FIGURE 3

Correlation between FGF23 changes and calcium-phosphorus product changes with and without treatment. In both the etelcalcetide and placebo groups, the changes in FGF23 correlate with changes in calcium phosphorus product (Etelcalcetide group: baseline to week 6, $\rho = 0.58$ and $p < 0.001$; baseline to week

12, ρ = 0.82 and p < 0.001. Placebo group: baseline to week 6, ρ = 0.59 and p = 0.001; baseline to week 12, ρ = 0.71 and p < 0.001.)

FIGURE 4

Correlation between CPP changes and calcium-phosphorus product changes with and without treatment. The changes in CPP are correlated in both the etelcalcetide and placebo groups, but the correlation is weaker in the etelcalcetide group. (Etelcalcetide group: baseline to week 6, ρ = 0.41 and p < 0.001; baseline to week 12, ρ = 0.56 and p <0.001. Placebo group: baseline to week 6, ρ = 0.56 and p < 0.001; baseline to week 12, ρ = 0.61 and p < 0.001.)

Table 1. Spearman correlation coefficients between percent change in FGF23 and CPPs and other bone-mineral markers

Bone mineral-markers	FGF23	CPP
Baseline to week 6		
Corrected calcium	0.42*	0.20
Phosphorus	0.48*	0.47*
Corrected calcium × Phosphorus	0.66*	0.47*
intact PTH	0.24	0.16
CPPs	0.29*	
Baseline to week 12		
Corrected calcium	0.47*	0.18
Phosphorus	0.63*	0.56*
Corrected calcium × Phosphorus	0.73*	0.58*
intact PTH	-0.005	-0.02
CPPs	0.56*	

Abbreviation: FGF23, fibroblast growth factor-23; CPP, calciprotein particle;

PTH, parathyroid hormone

Asterisks connote statistical significance of $p < 0.0028$