Author's response to reviews

Title: Fibroblast growth factor-23 and calcium phosphate product in young chronic kidney disease patients: A cross-sectional study

Authors:

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Author's response to reviews: see over
Dear Dr. Henderson,

Thank you for reviewing our manuscript, for your comments, and for the comments provided by the reviewers. Please, find below our responses to the reviewer’s concerns. For added clarity, we have first repeated the reviewer’s concerns, and then added our comment in *italics*, and highlighted the changes to the manuscript in **bold**. We hope that the revised manuscript will now be considered acceptable for publication.

**Reviewer(s)' Comments to Author:**

**Reviewer: 1**

Comments to the Author

The authors report a medium-size cohort of CKD patients and analyzed parameters of calcium-phosphate-metabolism in a cross-sectional design. Most -but not all – patients were children. The findings rather confirm earlier studies – FGF-23 associates with other Ca-P-parameter - than providing real novel results.

**Major issues:**

1. The cohort is very heterogeneous. I would strongly recommend deleting data from transplant patients and from dialysis patients. Numbers of these patients were much too low to allow a valid comparison to CKD-ND patients. Nonetheless, for a variety of reasons, these patients profoundly differ from patients with CKD without renal replacement therapy. Data from these patients should therefore not be lumped together.

*We appreciate this comment. With two reviewers citing concerns about the inclusion of renal transplant patients in our study, we repeated our statistical analysis excluding transplant recipients. The relationship between Cystatin C and serum FGF-23 levels remained statistically significant (Spearman r=0.41, p=0.0002). We were actually able to have a normal distribution when taking the log of FGF-23. The relationship between Cystatin C and serum FGF-23 levels was statistically significant (Pearson r=0.80, p<0.0001). Linear regression analysis also demonstrates statistically significant association between log-FGF-23 and the calcium phosphate product (Pearson r=0.62, p<0.0001). Former figures 3 and 4 are revised, and are now renamed Figure 1 and Figure 2 respectively to demonstrate these relationships.*

Re:  MS: 8033286026865782
Figure 1.
Title: The relationship between Cystatin C concentration and log(FGF-23) concentrations. Legend: There was a significant correlation (Pearson r=0.80, p<0.0001) between both markers that followed an exponential growth pattern. The regression line could be described as Y=1.389+0.23X.
Figure 2
Title: The relationship between FGF-23 concentrations and phosphate and Ca x P product.
Legend: There was a significant correlation between FGF-23 and both phosphate (Pearson r=0.7, p < 0.0001) and Ca x P (Pearson r=0.62, p<0.0001) that followed an exponential growth pattern. The regression lines could be described as Y=Y0*exp(k*X), with the equations for the regression line reading Y=3.425e^{-0.005} +0.7794 x X and Y=0.5119 + 1.502 x X, respectively.

The results section (page 6-8), as well as Tables 1 (page 19), 2 (page 20), 3 (page 22) and 4 (page 23), have been amended to reflect the new statistical analysis.

Concerns were also raised about the inclusion of patients on dialysis by one reviewer. The authors do acknowledge that the inclusion of such may introduce some bias, and we have included this in our discussion. However, the exclusion of such patients would have underpowered our study, and as a result, we have elected to include patients on dialysis in our statistical analysis and study.

2. There is severe redundancy in data presentation (compare Table 1 + Figure 1 as well as Table 4 + Figure 2)
We thank the reviewer for this recommendation. We have eliminated Figure 1 and Figure 2 to decrease redundancy in our manuscript.
3. Data presented in tables are not identical to data presented in the text, e.g. correlation cystatin C eGFR vs phosphate -0.277 (Table 3) vs -0.29 (Abstract). P 0.0025 vs 0.0048!
Maybe this discrepancy results from different statistical methods (correlation vs “one phase exponential association”); but then you must explain why you provide different statistical calculations. If you provide “one phase exponential associations” for univariate analysis, you should not use multivariate LINEAR regression analysis! Moreover, no relevant data on the multivariate regression analyses are provided (which variables are included? Stepwise / forward / backward? )

The reviewer is correct. We were able to find a normal distribution when log-transforming FGF-23. It became apparent to us that many manuscripts have chosen this approach, and that it would have been logical to do so given that we found a one-phase exponential relationship. This enabled us to use a linear approach. We altered the old figures 3 and 4 accordingly. In the abstract, we now listed the correlations between log(FGF-23) and the various parameters. For the analysis in table 3, we had to stick to univariate non-parametric analysis because of the distribution pattern of the variables. We decided to follow the reviewer’s suggestion to eliminate the multivariate analysis because of the challenges identified above.

Minor issues:
1. Please do not provide more than one decimal places in table 1.
2. Table 3 is very busy. There is no need to provide “P value summaries”. Again, you provide too many decimal places. For correlation coefficients, two decimal places are oK.

Thank you for these suggestions. We have amended Tables 1 (page 19) and 3 (page 22) as requested.

Reviewer: 2

This paper from Liu, Filler et al is interesting and well-written. Briefly, it reports bone and mineral metabolism in a cross-sectional study of 102 young CKD patients, describing for the first time in such a population a positive association between FGF23 and calcium/phosphate product.

Major comments
1. In the inclusion criteria, patients are ‘free of vascular calcifications’; however, can a single plain chest X-ray rule out the presence of vascular calcifications?

Thank you for this comment. The authors agree that a single plain chest x-ray cannot completely rule out the presence of vascular calcifications, but for the purposes of this study, the authors used the chest x-ray as a non-invasive, qualitative screen to exclude
patients with obvious aortic arch calcifications. To clarify this point, the manuscript now reads:

“The aim of the current study was to assess the relationship of FGF-23 with the Ca x P, CKD related mineral bone abnormalities, and the prevalence of therapies to correct them in a representative cohort of children and young adults with CKD who do not have obvious vascular calcifications on planar x-ray.”(page 5, lines 21-23, page 6, line 1)

- Moreover, were some vascular and cardiac data available in this cohort as well as growth data? They should be added in Table 2. **Kidney Disease: Improving Global Outcomes (KDIGO) does suggest that plain x-ray and echocardiography are appropriate alternatives to cardiac CT to detect vascular and valvular calcifications (1).** Unfortunately, we lacked a complete set of echocardiographic data on our study cohort; there was no other vascular or cardiac data available for this cohort. The authors thank the reviewer for the suggestion of adding growth data, and we have added in the average height z-scores and standard deviation as height z-scores were normally distributed.

2. A past of renal transplantation may be a confounding factor, and in Table 2 the proportion of patients receiving corticosteroids in each group should appear. Do you find an effect of corticosteroids (used both in glomerular diseases and in transplantation probably) on FGF23 and other parameters of mineral metabolism in your cohort?

The authors acknowledge that the past of renal transplantation may be a confounding factor, and as a result, have performed the statistical analysis again excluding all renal transplant recipients. **This analysis appears in the revised manuscript.** The relationship between Cystatin C and the log of serum FGF-23 levels remains statistically significant (Pearson r=0.80, p<0.0001). Linear regression analysis also demonstrates statistically significant association between log(FGF-23) and the calcium phosphate product (Pearson r=0.62, p<0.0001). Former figures 3 and 4 are revised, and are now renamed Figure 1 and Figure 2 respectively to demonstrate these relationships.

The authors thank the reviewer for the suggestion to include the proportion of patients receiving corticosteroids by CKD stage. **Table 2 (page 20) has been amended to include this information.** As we excluded the transplant patients, the main concern of the reviewer has been addressed. However, 6 of the non-transplant patients received steroids. The small number did not allow us to perform a priory analysis with and without steroids. We acknowledge that Bachetta et al (2) found that FGF-23 levels are elevated in children with steroids. **Our results suggest that GFR is perhaps the most important factor, and with only 1 patient with CKD 4/5 receiving steroids, this is impossible to analyze from our data.** However, we acknowledge this limitation in the discussion.
3. Table 2: the age in the different CKD group should appear, since phosphate levels are greatly influenced by age. Moreover, the phosphate levels should be expressed depending on age (for example in SDS using the references published by Carpenter); in Table 2, one normal level for serum phosphate when patients are aged between 2.5 and 27 years does not seem logical.

*The effect of age on serum phosphate levels is most substantial during infancy, between ages 0-2 years; the higher serum phosphorus concentration in infants can be attributed to an increased fractional phosphate reabsorption, and this may be further augmented by a low GFR (1). We therefore excluded patients under the age of 18 months; in fact, there were no patients in our cohort who were under 2 years of age. As a result, we decided to use one normal reference range for the study cohort.*

- The affirmation that FGF23 production increases in response to hyperphosphatemia during CKD should be taken with caution: in the results from the CKiD cohort, while FGF23 levels begin to increase at the early stages of CKD, there is an initial drop in serum phosphate level.

*Thank you for noting this. We have removed reference to FGF-23 increasing in response to hyperphosphatemia and rewritten those sentences as follows:*

“**FGF-23 is thought to be produced by altered osteocyte function in early CKD [3] and is elevated in patients with end-stage kidney disease**” *(page 5, lines 4-7)*

“The elevated FGF-23 levels observed in CKD have been explained by both increasing production by altered osteocyte function [3, 25] and by accumulation secondary to decreased renal clearance, as FGF-23 is a low molecular weight protein that is freely filtered across the glomeruli [13]” *(page 4, lines 23 – page 5, line 1)*

**Minor comments**

1. In the background end stage renal disease is abbreviated as CKD?
   *Again, thank you for catching this. The erroneous abbreviation has been removed.*

2. In the background, increased FGF23 levels when GFR decreases may be explained by a decreased renal clearance, but not only, and it should be clarified in the introduction and not later in the discussion.

*Thank you for the suggestion. We have rewritten the following sentence to provide more clarity around this concept:*

“In addition to increased FGF-23 production by osteocytes [3], FGF-23 concentrations may also rise because of accumulation in the serum secondary to decreased glomerular filtration. FGF-23 is a small molecular weight molecule,
similar to that of Cystatin C (CysC), which also accumulates in serum in patients with decreased renal clearance [13].” (Page 5, Lines 6-11)

3. At the end of the background ‘in children and young adults, where the confounding factors associated with later age are not yet present’: are not yet or are less present? See the paper published by Goodman and Salusky in the NEJM (that should also be referenced).

We revised the following sentence:
“However, there is no data on the relationship between FGF-23 and the calcium phosphate product (Ca x P) in children and young adults, where the confounding factors associated with later age are less present.” (page 5)

Thank you for the suggestion of this reference. We have also added in this reference in the following manner:

“In fact, children and young adult patients with calcifications on dialysis have higher serum phosphorus concentrations and a higher calcium–phosphorus ion product in serum [11].” (page 5, lines 18-20)

4. The PTH assay used for this study should be detailed.

Serum PTH concentrations were assayed by a solid-phase, two-site chemiluminescent enzyme-labelled immunometric assay (Immunlite 2000 Intact PTH from Diagnostic Products Corporation, Los Angeles, CA, USA). This was added to the manuscript. The paragraph now reads:
In addition to routine blood work that was regularly obtained for the monitoring of the CKD, we obtained serum for FGF-23 and CysC levels in each case. We also measured phosphate, calcium, ionized calcium, serum albumin and total protein, bicarbonate, vitamin D metabolites (1, 25-dihydroxy- and 25-hydroxyvitamin D), and intact PTH levels, and urinary calcium to creatinine ratio, using standard laboratory tests. Cystatin C eGFR was calculated using the “Filler-formula” [26, 27]. Serum PTH concentrations were assayed by a solid-phase, two-site chemiluminescent enzyme-labelled immunometric assay (Immunlite 2000 Intact PTH from Diagnostic Products Corporation, Los Angeles, CA, USA). Ca x P was the simple product of the total serum calcium and the serum phosphate. (page 17, lines 17-20).

5. Another limitation should be discussed in addition to the absence of urinary phosphate excretion: the absence of nutritional assessment of phosphate intake.

The authors thank the reviewer for this suggestion. The following sentence has been added to the manuscript:
“We also did not assess patients on their dietary phosphorus intake, which can contribute to phosphorus retention and bone mineral disorder with decreasing GFR.” (page 10, lines 17-19)
We believe that we have adequately addressed the reviewers’ concerns and wish to resubmit the manuscript for your kind consideration.

Sincerely

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References:
