PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Associations of Fibroblast Growth Factor 23, Vitamin D and Parathyroid hormone with 5 Year Outcomes in a Prospective Primary Care Cohort of People with Chronic Kidney Disease Stage 3
AUTHORS	Shardlow, Adam; McIntyre, Natasha; Fluck, Richard; McIntyre, CW; Taal, Maarten

VERSION 1 - REVIEW

REVIEWER	Geoffrey A Block, MD Denver Nephrology Research Division USA
REVIEW RETURNED	13-Mar-2017

GENERAL COMMENTS	This is an extraordinarily well written manuscript describing a very relevant and important issue. In general my concerns, while minor, do affect the reporting and conclusions that the authors articulate. 1. The title suggests causation by using the term "Effect of..." and none of these observations reach the level of causation I would suggest the title be changed to make it clear they are describing an association between X, Y and Z. 2. I believe the definition of 'progression' should be stated in the methods as an outcome measure rather than what was 'not' used as a definition. 3. My primary (and significant) concern relates to the population studied which appears to have CKD but perhaps driven substantially by their age rather than by their disease (so called age-related decline in eGFR due to the importance of age in the formula). This is tremendously important given that their baseline characteristics for these parameters are not consistent with other reports of patients with this level of reduced eGFR. This is very problematic as it affects the outcomes studied and the relationship, generally, between the variables and the outcomes. One way to explore this further is to show us explicitly the progression of CKD of these participants. I suspect that the vast majority had no change beyond that associated with a change in age (i.e. the expected change when using this formula). If true, the authors will certainly need to done down any conclusions about the variables of interest and their potential relationship to the outcomes. Only 0.2% progressed to ESKD over 5 years suggesting this really remarkably different comorbidity. The conclusions, while different are still important- 25D and PTH do seem to predict important outcomes EVEN among those not particularly at risk for progressive CKD -a remarkable finding. Thus the findings will be relevant to a primary care physician but not, perhaps to a nephrology population with 'true' stage 3 CKD. In addition to the actual progression over time I'd like to see the numbers of those who had stage 3B and I'd like to see the population shown using the current KDIGO staging classification

	using eGFR and ACR. A table showing the N within each stage/category would be tremendous. 4. Less than 350 individuals had a GFR < 45 and I worry about drawing any sweeping conclusions from an observational study with such low N's. The authors do indeed discuss these limitations in the discussion but do not, ultimately, let it dissuade them from extrapolating their conclusions to all patients with stage 3 CKD and that is the aspect that troubles me. I'm not sure why , in the conclusion the authors limit their supposition about detection and treatment to vitamin D and do not include PTH. As inferred above I do not support the broad discounting of the utility of FGF23 as a risk marker in those with early CKD- this may be true among patients at low risk of progression and the numbers of participants at greater risk (< 45) may have been too small given that almost no one actually reached ESKD. Overall I find the manuscript to be an important contribution to the field and I think it will be even stronger with these few modifications.

REVIEWER	Kazuhiko Tsuruya Kyushu University, Japan
REVIEW RETURNED	13-Mar-2017

GENERAL COMMENTS	To assess whether FGF23 is similarly a risk factor in people with early CKD, and how this risk compares to that associated with vitamin D deficiency or elevated PTH, the authors conducted a prospective cohort study in 1,664 subjects with CKD stage 3. The endpoints are all cause mortality and progression of CKD. The results showed that vitamin D deficiency and elevated PTH were independent risk factors for all-cause mortality but elevated FGF23 was not. Thus, the authors concluded that FGF23 may not be as important in early CKD. The theme of this study is intriguing and the manuscript is well written; however, there are some concerns to be addressed. 1. The authors described that KDIGO criteria was used as a definition of CKD progression which is defined as a 25% decline in GFR, accompanied by a worsening of GFR category, or a worsening of albuminuria category. However, worsening of albuminuria category is not included in KDIGO criteria (Kidney Int Suppl, 2012) and I agree with the definition without worsening of albuminuria category. Thus, I recommend the authors to change the definition of CKD progression from the definition with worsening of albuminuria category into the definition without it. 2. The authors separated the participants into 4 groups according to the level of FGF23, PTH, or 25(OH)Vit D. Based on what evidence, did the authors determine the cut-off points for these separations? The authors should explain this point in detail. 3. In the sensitivity analyses, stratified analysis was conducted to compare the all-cause mortality between people with CKD 3a and those with CKD 3b, and between people aged less than 75 at baseline and those aged over 75. In the stratified analyses, the authors should statistically examine the interaction between the 2 groups.

	4. Spelling errors should be corrected. Page 2, line 13; focussed → focused Page 12, line 31; above 65 pg/mL → above 95 pg/mL

REVIEWER	Darryl Quarles Univeristy of Tennessee Health Science Center (UTHSC) USA
REVIEW RETURNED	20-Mar-2017

GENERAL COMMENTS	This is a most important paper that re-assesses the role of FGF-23 in mortality in CKD. It overcomes the limitations of prior studies by being prospective and including other measures, such as vitamin D and PTH, that can co-vary with FGF-23 and which might also impact outcomes. They find that FGF-23 does not account for increased mortality when considered in the context of changes in PTH and vitamin D. This careful analysis counters the prevailing "rush to judgement" by other investigators who have placed so much importance on FGF-23 without adequate data to support their beliefs. Some important caveats should be noted. The severity of the renal failure and degree of FGF-23 elevations were mild. Possibly much higher FGF-23 levels are needed to exert cardio-toxicity. FGF-23 is associated with defects in innate immunity. Was there any relationship between elevations of FGF-23 and decreased Vitamin D and infectious outcomes? What were the hazard rations for infectious mortality? FGF-23 is associated with sodium retention, low aldosterone and hypertension in mice. Did they observe any relationship between FGF-23 and renal sodium handling or BP? Shouldn't repeated measures of FGF-23, vitamin D and PTH be measured during the study, rather than only at the beginning. Could progressive elevations of FGF23 modify the results? The authors should be more bold in their discussion. There negative findings have significant implications. They state that "The reasons for these variable results are not clear, but may relate to differences between the study populations and methodology"; and while this is one possibility, the other is that modest elevations of bioactive FGF-23 are not associated with increased mortality in CKD 3, and if so, maybe the focus should be on treating Vitamin D deficiency and suppressing PTH. The negative clinical studies on vitamin D supplementation and mortality need to be mentioned, as well as the effects of vitamin D treatment to increase FGF-23. Also, some mention of the difficulty in using calcimimetics in CKD should be mentioned (increases serum phosphorus).

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

1. The title suggests causation by using the term "Effect of..." and none of these observations reach the level of causation I would suggest the title be changed to make it clear they are describing an association between X, Y and Z.

Response: We accept the reviewer's comments and have suggested a change in title to 'Associations of Fibroblast Growth Factor 23, Vitamin D and Parathyroid hormone with 5 Year Outcomes in a Prospective Primary Care Cohort of People with Chronic Kidney Disease Stage 3'

2. I believe the definition of 'progression' should be stated in the methods as an outcome measure rather than what was 'not' used as a definition.

Response: We agree and have changed this is the abstract. We have expanded on the definition used for CKD progression in the methods section.

3. My primary (and significant) concern relates to the population studied which appears to have CKD but perhaps driven substantially by their age rather than by their disease (so called age-related decline in eGFR due to the importance of age in the formula). This is tremendously important given that their baseline characteristics for these parameters are not consistent with other reports of patients with this level of reduced eGFR. This is very problematic as it affects the outcomes studied and the relationship, generally, between the variables and the outcomes. One way to explore this further is to show us explicitly the progression of CKD of these participants. I suspect that the vast majority had no change beyond that associated with a change in age (i.e. the expected change when using this formula). If true, the authors will certainly need to done down any conclusions about the variables of interest and their potential relationship to the outcomes. Only 0.2% progressed to ESKD over 5 years suggesting this really remarkably different comorbidity. The conclusions, while different are still important- 25D and PTH do seem to predict important outcomes EVEN among those not particularly at risk for progressive CKD -a remarkable finding. Thus the findings will be relevant to a primary care physician but not, perhaps to a nephrology population with 'true' stage 3 CKD. In addition to the actual progression over time I'd like to see the numbers of those who had stage 3B and I'd like to see the population shown using the current KDIGO staging classification using eGFR and ACR. A table showing the N within each stage/category would be tremendous.

Response: We agree with the reviewer that our study population is different (and at much lower risk of CKD progression) to that included in previous studies that have examined FGF23 as a risk factor in CKD population, in particular the CRIC study. These important differences are discussed in detail in the Discussion section of the paper. In addition we have strengthened this message by emphasizing in the abstract and conclusions that our observations relate to a low risk population in primary care and may therefore not be applicable to higher risk populations in secondary care. As suggested, we have added a supplementary table showing the numbers of participants according to KDIGO GFR and albuminuria categories. We have also added some data regarding the incidence of CKD progression. Detailed information regarding CKD progression in this cohort has previously been published (see reference 20).

4. Less than 350 individuals had a GFR < 45 and I worry about drawing any sweeping conclusions from an observational study with such low N's. The authors do indeed discuss these limitations in the discussion but do not, ultimately, let it dissuade them from extrapolating their conclusions to all patients with stage 3 CKD and that is the aspect that troubles me. I'm not sure why , in the conclusion the authors limit their supposition about detection and treatment to vitamin D and do not include PTH.

As inferred above I do not support the broad discounting of the utility of FGF23 as a risk marker in those with early CKD- this may be true among patients at low risk of progression and the numbers of participants at greater risk (< 45) may have been too small given that almost no one actually reached ESKD.

Response: As stated above, we agree that our study population was at low risk and included a relatively low proportion with CKD G3b. We believe that this is clear in the manuscript and we have strengthened the message about our data applying only to low risk cohorts as discussed above. We focussed on vitamin D deficiency because this is easily correctable with supplementation. We have expanded our comments in the discussion regarding the association with PTH.

Reviewer 2

The authors described that KDIGO criteria was used as a definition of CKD progression which is defined as a 25% decline in GFR, accompanied by a worsening of GFR category, or a worsening of albuminuria category. However, worsening of albuminuria category is not included in KDIGO criteria (Kidney Int Suppl, 2012) and I agree with the definition without worsening of albuminuria category. Thus, I recommend the authors to change the definition of CKD progression from the definition with worsening of albuminuria category into the definition without it.

Response: We agree that albuminuria is not included in the KDIGO criteria for progression in Section 2.1.3 but the guideline does state under “Clarification of Issues and Key Points” in Chapter 2 that “Assessment of both GFR and albuminuria should be undertaken to evaluate progression.” We therefore thought that it was appropriate to include progression of albuminuria in our definition in order to include all evidence of worsening CKD. Additionally, given that we have not shown any significant associations with CKD progression using our definition based upon eGFR and albuminuria changes, we would be very unlikely to show any association using a definition using only eGFR which would reduce the number of outcome events.

2. The authors separated the participants into 4 groups according to the level of FGF23, PTH, or 25(OH)Vit D. Based on what evidence, did the authors determine the cut-off points for these separations? The authors should explain this point in detail.

Response: For vitamin D we used clinically relevant published thresholds for vitamin D deficiency (<25nmol/L), insufficiency (25-49nmol/L) and “optimal” levels (>75nmol/L) (see reference 19) For FGF23 and PTH we used previously published thresholds to define elevated values and chose the other cut points to yield four groups of reasonable size. This has now been clarified in the manuscript.

. In the sensitivity analyses, stratified analysis was conducted to compare the all-cause mortality between people with CKD 3a and those with CKD 3b, and between people aged less than 75 at baseline and those aged over 75. In the stratified analyses, the authors should statistically examine the interaction between the 2 groups.

Response: Thank you for this helpful comment. There was no interaction between age ≥75y and CKD 3a/3b. We have added this to the results.

4. Spelling errors should be corrected.
Page 2, line 13; focussed → focused
Page 12, line 31; above 65 pg/mL → above 95 pg/mL

Response: The first has been corrected but “above 65pg/mL” is correct.

Reviewer 3

The severity of the renal failure and degree of FGF-23 elevations were mild. Possibly much higher FGF-23 levels are needed to exert cardio-toxicity.

Response: We agree with the reviewers comment and have discussed this in the manuscript.

FGF-23 is associated with defects in innate immunity. Was there any relationship between elevations of FGF-23 and decreased Vitamin D and infectious outcomes? What were the hazard rations for infectious mortality?

Response: The number of deaths due to infection was low (n=61) and we therefore lacked statistical power to investigate this.

FGF-23 is associated with sodium retention, low aldosterone and hypertension in mice. Did they observe any relationship between FGF-23 and renal sodium handling or BP?

Response: We agree that this would be interesting but we do not have detailed data on sodium handling in this cohort. We have previously reported that there was no relationship between FGF23 and systolic BP and a weak negative association with diastolic BP at baseline (see reference 11)

Shouldn't repeated measures of FGF-23, vitamin D and PTH be measured during the study, rather than only at the beginning. Could progressive elevations of FGF23 modify the results?

Response: We agree that this would provide interesting additional information but at present we have only baseline data.

The authors should be more bold in their discussion. There negative findings have significant implications. They state that "The reasons for these variable results are not clear, but may relate to differences between the study populations and methodology"; and while this is one possibility, the other is that modest elevations of bioactive FGF-23 are not associated with increased mortality in CKD 3, and if so, maybe the focus should be on treating Vitamin D deficiency and suppressing PTH.

Response: Thank you. We have had to balance this comment with those of Reviewer #1 who requested that we “tone down” our conclusions. We believe that we have achieved a good balance by stating that whereas FGF23 was not associated with adverse outcomes in our low risk cohort, it may be a useful prognostic marker and therapeutic target in higher risk cohorts. We agree that the focus should be on vitamin D deficiency in primary care and have stated this in the manuscript.

The negative clinical studies on vitamin D supplementation and mortality need to be mentioned, as well as the effects of vitamin D treatment to increase FGF-23. Also, some mention of the difficulty in using calcimimetics in CKD should be mentioned (increases serum phosphorus).

Response: We have mentioned the lack of evidence of benefit from vitamin D supplementation as

suggested. We have not added comments relating to calcimimetics because we feel they would not be used in this population.

VERSION 2 – REVIEW

REVIEWER	Kazuhiko Tsuruya Kyushu University, Japan
REVIEW RETURNED	21-Jun-2017

GENERAL COMMENTS	I have no further comment.

REVIEWER	Darryl Quarles Uthsc Usa
REVIEW RETURNED	18-Jun-2017

GENERAL COMMENTS	Sent previously