ANSWERING REVIEWERS

Dear Editor,

Please find enclosed the edited manuscript in word format (file name: 22395-Review.doc).

Title: Role of calcium in polycystic kidney disease: From signaling to pathology

Author: Alessandra Mangolini, Lucia de Stephanis, Gianluca Aguiari

Name of Journal: *World Journal of Nephrology*

ESPS Manuscript NO: 22395

The manuscript has been improved according to the suggestions of reviewers:

Reviewer 1 code: 00503002 (the text changes are marked in yellow).
Comments: This is a well written minireview on the current understanding of the role of calcium channels and intracellular calcium signalling in the pathogenesis of ADPKD. The only minor comment is that in figure 2A (the western blot) needs to be mentioned so that the reader knows what this is showing.

Answer: As suggested by the Reviewer, the legend of figure 2A was modified as follows:

Figure 2: Downregulation of *PKD1* and *PKD2* genes increases FBS-induced calcium oscillations in HEK293 cells.
A: The stable transfection of HEK293 cells with plasmids containing specific anti-PKD1 and anti-PKD2 sequences causes a partial (+/-) or complete (-/-) downregulation of PC1 and PC2 expression compared with HEK293 cells stably transfected with scramble sequences (Control). *PKD1* and *PKD2* gene silencing was evaluated by Western blotting using anti-PC1 and anti-PC2 antibodies. Calcium oscillations were increased in both partially (+/-) and fully (-/-) cells silenced for the *PKD1* gene, as well as in fully (-/-) *PKD2*-silenced cells, as compared with scramble-treated cells (control). The number of oscillations/15 min were: 12±1.5 in $PKD1^{(+/-)}$ cells, 12.2±1.42 in $PKD1^{(-/-)}$ cells and 11.13±1.79 in $PKD2^{(-/-)}$ cells, vs. 6.39±1.09 in control cells (**P<0.01; *P<0.05). B: The expression of full-length exogenous PC2 fused with GFP in $PKD2^{(-/-)}$ cells restores normal calcium oscillations (11.13±1.79 oscillations/15 min in $PKD2^{(-/-)}$ cells vs. 7.72±1.07 in $PKD2^{(-/-)}$ cells transiently transfected with PKD2-GFP cDNA; *P<0.05). Western blotting, oscillation recording and cell imaging were performed as previously reported[16]. Data, obtained from three different experiments analyzing at least 45 cells for every HEK293 clone, are represented as mean ± standard deviation. Analysis of data was performed using Student's t test, and differences were considered significant at a value of P<0.05. PKD1, polycystic kidney disease 1; PKD2, polycystic kidney disease 2; HEK293; human embryonic kidney cells; GFP, green fluorescent protein.

Reviewer 2 code: 00503254

Comments: None.

Reviewer 3 code: 00149476 (the text changes are marked in yellow).

Comments: This is a clear and nicely written brief review of the role of calcium in PKD. Major Comment:
1. The legend to figure 2 does not really explain the figure. Panel A shows a western blot but this is never mentioned in the legend.
2. The size markers are also missing. Please clarify the legend to better explain the figure. Minor Comment:
3. Page 4, 3 lines from bottom - chances should be changes.

Answer 1: As suggested by the Reviewer the legend of figure 2A was modified as follows:

Figure 2: Downregulation of *PKD1* and *PKD2* genes increases FBS-induced calcium oscillations in HEK293 cells.
A: The stable transfection of HEK293 cells with plasmids containing specific anti-PKD1 and anti-PKD2 sequences causes a partial (+/-) or complete (-/-) downregulation of PC1 and PC2 expression compared with HEK293 cells stably transfected with scramble sequences (Control). *PKD1* and *PKD2* gene silencing was evaluated by Western blotting using anti-PC1 and anti-PC2 antibodies. Calcium oscillations were increased in both partially (+/-) and fully (-/-) cells silenced for the *PKD1* gene, as well as in fully (-/-) *PKD2*-silenced cells, as compared with scramble-treated cells (control). The number of oscillations/15 min were: 12±1.5 in $PKD1^{(+/-)}$ cells, 12.2±1.42 in $PKD1^{(-/-)}$ cells and 11.13±1.79 in $PKD2^{(-/-)}$ cells, vs. 6.39±1.09 in control cells (**P<0.01; *P<0.05). B: The expression of full-length exogenous PC2 fused with GFP in $PKD2^{(-/-)}$ cells restores normal calcium oscillations (11.13±1.79 oscillations/15 min in $PKD2^{(-/-)}$ cells vs. 7.72±1.07 in $PKD2^{(-/-)}$ cells transiently transfected with PKD2-GFP cDNA; *P<0.05). Western blotting, oscillation recording and cell imaging were performed as previously reported[16]. Data, obtained from three different experiments analyzing at least 45 cells for every HEK293 clone, are represented as mean ± standard deviation. Analysis of data was performed using Student's t test, and differences were considered significant at a value of P<0.05. PKD1, polycystic kidney disease 1; PKD2, polycystic kidney disease 2; HEK293; human embryonic kidney cells; GFP, green fluorescent protein.

Answer 2: As suggested by the Reviewer the size of markers were inserted in the new figure 2A.

Answer 3: The wrong word "chances" was substituted with "changes".