Reviewer's report

Title: TRPP2 dysfunction decreases ATP-evoked calcium, induces cell aggregation and stimulates proliferation in T lymphocytes

Version: 1 Date: 22 Apr 2019

Reviewer: Isabelle Meyts

Reviewer's report:

This is an interesting manuscript on the role of T cell Ca metabolism in ADPKD disease manifestations. However, there are multiple major and minor concerns with this paper as listed below. The main concern is that the conclusions are not supported by the the data though the data in itself are interesting. The entire manuscript also needs careful restructuring and the methodology needs some corrections and controls.

Abstract
Page 2:
Line 13: ADPKD is linked to the mutation … should be 'to mutations in either …'
Line 23: It is not very clear with what kind of cells the experiments are performed. In Methods section it is explained that T lymphoblast cells are produced but further more it says that "T lymphocytes" are used in the experiments. It should be very clear in the entire manuscript if the experiments are performed with T cell blasts or primary isolated T cells as conclusions are likely to differ.

Line 30 Why is the CFSE Flow method not used for T cell proliferation experiments?

Line 35 Please mention the exact mutation in PKD2.

Line 37 Traditionally a peptide is a molecule that consists of between 2 to 50 AA whereas proteins are made up of 50 or more AA. Please use the correct term in the manuscript. Use truncated protein as a result of a premature stopcodon.

Line 54 Replace sentence to Similar to observation made in kidney cells, mutations in PKD1 and PKD2 dysregulates ERK, mTOR and NFKB signaling in T lymphoblast cells.

Page 3:
Conclusion of abstract: start the conclusion about the new obtained results in the T lymphoblasts before making assumptions about the impact on the disease and potential disease targeted treatments.

In general the background need to be rewritten and take out the summary of your results (pag 4).
e.g. Autosomal dominant polycystic kidney disease (ADPKD) is the result of mutations in one allele of the PKD1 or PKD2 genes, followed by "second hit" somatic mutations of the other allele in renal tubule cells
= not mentioned in the manuscript

Pag 5:
Line 51: replace by PBL and neutrophil isolation and T lymphoblast culture
Line 53-65: replace this part by peripheral blood lymphocytes (PBLs) were isolated by lympholyte-H cell separation density gradient.

Pag 6
Line 5-7: How long is the PHA stimulation time for T lymphoblast cell preparation?
Line 27: How is the purity of the neutrophils measured?
Line 36: Provide company information of the used siRNA plasmid

Pag 9:
Results:
Line 22-28: should be moved to line 40 as second part of the expression experiment (B)
Line 28: rephrase sentence with the clinical data of the ADPKD cohort as summarized in supplemented Table 1.
Line 32-35: Please also mention the HEK293 and EBV LCL cultures in the methods.

Legends of figure 1 (pag 22)
Line 42-45: number of repeats of the data presented in A. Is this the best picture available? Is the difference between the kidney cells and the lymphoblasts significant?

Line 40-41: remove "the" from the R872X mutation
Use truncated protein instead of mutated peptide (also in legends)

Line 50-54: incorrect. Your western blotting only shows you the presence of reduced protein in the conditions where the R782X mutated T lymphoblasts cells of the patients are used. It is not suggesting anything about correct protein folding and downregulation of TRPP2 protein expression. In my opinion, you only can comment that the presence of the mutation has maybe in impact on the stability of your investigated protein.

Line 55-60 and pag 10 lines 1-15: new paragraph? Does not belong under protein expression data.

Discussion:
Pag 12
Line 54: the authors do not prove a dominant negative effect of the mutated protein

Page 13:
Line 14-54: All data should be in the results section.
I advise: starting from Line 58: start with what is already known in the kidney cells. Then explain your new data in T lymphoblasts and then explain the potential impact.

Pag 14.
Chemotaxis of neutrophils is in basal conditions of culture (BSA). It is hard to follow than in the discussion the section about the hypothesis that the neutrophil chemotaxis is driven by MIF. Can they measure MIF in their cultures? Short co-cultures of neutrophils and T lymphocytes may give more information
Are you sure that T lymphoblast cell aggregates are not just the result of increased proliferation ratio? T lymphoblast prefer to be in cell clumps.

Please confirm that you have included your review in the ‘Comments to Author’ box?
As a minimum standard, please include a few sentences that outline what you think are the authors' hypothesis/objectives, their main results, and the conclusions drawn. Your report should constructively instruct authors on how they can strengthen their paper to the point where it may be acceptable for publication, or provide detailed reasons as to why the manuscript does not fulfill our criteria for consideration. Please supply appropriate evidence using examples from the manuscript to substantiate your comments. Please break your comments into two bulleted or numbered sections: major and minor comments.

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- Is the exact sample size (n) reported for each experimental group/condition (as a number, not a range)?

- Are the description of any error bars and probability values appropriate?

- Are all error bars defined in the corresponding figure legends?

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