PQLC2 recruits the C9Orf72 complex to lysosomes in response to cationic amino acid starvation

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Review Timeline:

| Event                        | Date       |
|------------------------------|------------|
| Submission Date              | 2019-06-13 |
| Editorial Decision           | 2019-07-16 |
| Revision Received            | 2019-10-07 |
| Editorial Decision           | 2019-10-11 |
| Revision Received            | 2019-10-17 |

Monitoring Editor: Xiaochen Wang

Scientific Editor: Tim Spencer

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

DOI: https://doi.org/10.1083/jcb.201906076
July 16, 2019

Re: JCB manuscript #201906076

Dr. Shawn M Ferguson
Yale University School of Medicine
Department of Cell Biology
295 Congress Ave BCMM254E
New Haven, CT 06510

Dear Dr. Ferguson,

Thank you for submitting your manuscript entitled "PQLC2 signals lysosomal cationic amino acid abundance to the C9orf72 complex". The manuscript was assessed by expert reviewers, whose comments are appended to this letter. We invite you to submit a revision if you can address the reviewers' key concerns, as outlined here.

You will see that while both reviewers are supportive of the work, they each raise a number of concerns that will need to be addressed before the paper would be deemed suitable for publication at JCB.

As indicated in her/his review below, reviewer #1 would like you to demonstrate the functional relevance of PQLC2-C9orf72 interaction on lysosome function and/or signaling. To do this, Rev#1 suggested to test whether loss of PQLC2, 1) affects lysosome function (point #2) and 2) affects mTOR signaling, despite the fact that C9orf72 may act independent of the mTOR pathway (point #1). I agree that both of these points are important and should be tested in order to demonstrate not only the presence of the PQLC2-C9orf72 axis but also the functional importance of this pathway, as well as its relationship with the mTOR pathway. Given that the authors already generated PQLC2 KO cells, it should be straightforward to test whether mTOR signaling is affected in KO cells under amino acid depletion and repletion conditions. Similarly, lysosome pattern and activity can be examined in PQLC2 KO cells at both normal and starvation conditions to show whether lysosome function is impaired by PQLC2 KO. Reviewer #1 also feels that it is necessary to determine whether lysosomal-targeted WDR41/C9orf72/SMCR8 can rescue lysosomal function in the PQLC2 KO cells (point #3) - and we agree that this would be an important addition. However, while examination of the domains of WDR41 which mediate the interaction with PQLC2 and C9orf72/SMCR8 would be an intriguing avenue of exploration, we do not believe that it would be necessary to support the main conclusions of the study and so we will not require this to be addressed in the revision.

We also hope that you will be able to address the single point raised by reviewer #2 with new data.

While you are revising your manuscript, please also attend to the following editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

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The typical timeframe for revisions is three to four months; if submitted within this timeframe, novelty will not be reassessed at the final decision. Please note that papers are generally considered through only one revision cycle, so any revised manuscript will likely be either accepted or rejected.

When submitting the revision, please include a cover letter addressing the reviewers' comments point by point. Please also highlight all changes in the text of the manuscript.

We hope that the comments below will prove constructive as your work progresses.

Thank you for this interesting contribution to Journal of Cell Biology. You can contact us at the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Sincerely,

Xiaocheng Wang, PhD
Monitoring Editor
JCB

Tim Spencer, PhD
Deputy Editor
Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

The manuscript by Amick J. et al identified the lysosomal basic amino acid transporter PQLC2 as an essential factor for lysosomal recruitment of the C9orf72 complex. Using IP-mass spectrometry they found that PQLC2 associated with the lysosome-targeted C9orf72. They further demonstrated that PQLC2 is necessary and sufficient to recruit the C9orf72 complex to the lysosome. In addition, they identified that the PQ loop is important for PQLC2 to interact with the C9orf72 complex, and
that WDR41 mediates the interaction of the C9orf72 complex with PQLC2. Moreover, they found that the PQLC2 substrates, arginine, lysine, and histidine, negatively regulate the interaction between PQLC2 and the C9orf72 complex, which is a new amino acid sensing mechanism distinct from the Rag GTPase-mediated amino acid sensing and mTOR activation.

In general, the data presented in this paper clearly and convincingly demonstrated that PQLC2 is responsible for lysosomal recruitment of the C9orf72 complex, and revealed a new mechanism for lysosomal sensing of amino acids. The paper will be benefited from addressing the following questions regarding the functional interaction of PQLC2 with the C9orf72 complex.

1. Does PQLC2 knockout affect mTOR signaling on its own or SLC38A9-mediated mTOR signaling?

2. It was shown that loss of LAAT-1, the PQLC2 homolog in C. elegans, impairs lysosomal functions. Does PQLC2 knockout affect lysosomal functions in mammalian cells?

3. If PQLC2 knockout cells are defective in lysosomal function or mTOR signaling, whether lysosomal targeted WDR41/C9orf72/SMCR8 could rescue such defects?

4. It would be nice to determine which functional domains of WDR41 mediate the interaction with PQLC2 and C9orf72/SMCR8.

Reviewer #2 (Comments to the Authors (Required)):

The protein encoded by the frontotemporal degeneration-amyotrophic lateral sclerosis gene C9orf72 forms a multisubunit complex which is required for lysosomal function and is recruited to the lysosomal surface upon amino acid starvation. In this study, the authors identify the lysosomal cationic amino acid transporter PQLC2 as a key protein in this process. PQLC2 physically interacts with the C9orf72 complex, essentially through its WDR41 subunit; it is necessary and sufficient to recruit the complex at the lysosomal surface; and it signals cationic amino acid sufficiency (or scarcity) to the C9orf72 complex.

In an elegant experiment, the authors took advantage of the role of PQLC2 in the cysteamine treatment of cystinosis to acutely induce a lysine-like PQLC2 substrate in the lysosomal lumen. This treatment released the C9orf72 complex from lysosomes, thus strongly suggests that the PQLC2/C9orf72 axis senses intralysosomal amino acids.

This is a remarkable study, which improves our understanding of the C9orf72 complex and unveils a novel amino acid-sensing mechanism at the lysosome which is distinct from previously known amino acid-sensing mechanisms.

My only concern regards the interpretation of the P55L/P201L mutant effects. The authors argue that these mutations disrupt C9orf72 complex recruitment through conformational effects. This is quite possible as, in distantly related transporters with similar PQ motifs, the prolines act as hinges for structural transitions. However, the low intensity of the P55L/P201L band in Fig. 3B suggests another more trivial explanation: the poor targeting of the P55L/P201L mutant to the lysosome. The immunofluorescence data in Fig S3 are used to argue that lysosomal targeting is not affected. However such images are less quantitative than Western blots.

This is otherwise an excellent study.
Response to Reviewers

Reviewer #1

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In general, the data presented in this paper clearly and convincingly demonstrated that PQLC2 is responsible for lysosomal recruitment of the C9orf72 complex, and revealed a new mechanism for lysosomal sensing of amino acids. The paper will be benefited from addressing the following questions regarding the functional interaction of PQLC2 with the C9orf72 complex.

We are grateful for the time and effort that went into this review as well as for the overall positive evaluation.

1. Does PQLC2 knockout affect mTOR signaling on its own or SLC38A9-mediated mTOR signaling?

In response to this question, we have performed new experiments. The results of these experiments are presented in Figure 8. We observed that PQLC2 KO cell have an impaired ability to activate mTORC1 signaling in response to arginine, lysine and histidine, the cationic amino acid substrates of PQLC2. This defect is specific as it is reversed upon re-expression of PQLC2 in the KO cells.

Although the relationship between PQLC2 and SCL38A9 in coordinating lysosomal responses to amino acids availability will be of potential interest for future studies, this represents a separate and very complex topic that is distinct from the focus of our current manuscript on the newly discovered role for PQLC2 in the regulated recruitment of the C9orf72 complex to lysosomes.

We also note that there is still uncertainty about the precise role(s) for SLC38A9 in the transport of lysosomal amino acids as it has been proposed to have multiple functions including: an arginine regulated exporter of leucine and other essential amino acids from lysosomes (Wyant et al, Cell, 2017); a sensor of lysosomal cholesterol (Castellano et al, Science, 2017); a lysosomal arginine transporter (Wang et al, Science, 2015); and a transporter for lysosomal glutamine, arginine and asparagine (Rebsamen et al, Nature, 2015). Thus, although these high-profile publications demonstrate that there is great interest in SLC38A9, there is not yet a consensus on the precise physiological function of Slc38A9 at lysosomes. This uncertainty further supports our conclusion that exploration of SLC38A9 is beyond the scope of our current manuscript.
2. It was shown that loss of LAAT-1, the PQLC2 homolog in C. elegans, impairs lysosomal functions. Does PQLC2 knockout affect lysosomal functions in mammalian cells?

We have not observed evidence of a generalized impairment in lysosome function in PQLC2 KO cells. In support of this, we present new data in Figure 8 that there is no major defect in lysosome function/proteolysis in PQLC2 KO cells as revealed by the normal maturation of multiple cathepsins and the ability of lysosomes to keep up with the clearance of LC-3 that is delivered via the autophagy pathway.

3. If PQLC2 knockout cells are defective in lysosomal function or mTOR signaling, whether lysosomal targeted WDR41/C9orf72/SMCR8 could rescue such defects?

Following up on the new data relating to point #1, in Figure 8, we present the results of new experiments wherein we have expressed a constitutively lysosome targeted form of C9orf72 in PQLC2 KO cells and have found that it helps to restore the ability cationic amino acids to activate mTORC1 signaling.

4. It would be nice to determine which functional domains of WDR41 mediate the interaction with PQLC2 and C9orf72/SMCR8.

We agree that it will be important to eventually understand how WDR41 is able to form a bridge between PQLC2 and C9orf72/SMCR8. However, (in agreement with the editorial summary) addressing such mechanisms in detail raises a host of questions that extend beyond the major message of this current manuscript.

Reviewer #2

The protein encoded by the frontotemporal degeneration-amyotrophic lateral sclerosis gene C9orf72 forms a multisubunit complex which is required for lysosomal function and is recruited to the lysosomal surface upon amino acid starvation. In this study, the authors identify the lysosomal cationic amino acid transporter PQLC2 as a key protein in this process. PQLC2 physically interacts with the C9orf72 complex, essentially through its WDR41 subunit; it is necessary and sufficient to recruit the complex at the lysosomal surface; and it signals cationic amino acid sufficiency (or scarcity) to the C9orf72 complex.

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This is otherwise an excellent study.

We deeply appreciate this highly positive evaluation of our manuscript!

We have carefully examined the subcellular localization of the PQLC2 P55L/P201L mutant and conclude that there is no detectable defect in the targeting of this protein to lysosomes and thus remain confident in our original conclusions regarding the inability of this mutant to interact with the C9orf72 complex. We have updated the manuscript in the following ways to solidify this conclusion.

First, we now show that the defective ability of the P55L/P201L mutant to recruit C9orf72/SMCR8 to lysosomes is not due to a lysosome trafficking defect of the P55L/P201L mutant as this difference is still apparent even after normalizing lysosomal C9orf72 and SMCR8 to the levels of lysosomal PQLC2 (Figure 3D).

Second, we have performed a new quantitative analysis of subcellular localization for wild type PQLC2 and the P55L/P201L mutant (Figure S1A, S3B-F) which showed that both proteins exhibit near identical degrees of enrichment on LAMP1-positive late endosomes and lysosomes. Conversely, they both show the same minimal degree of co-enrichment with calnexin, a marker of the endoplasmic reticulum.

These 2 independent sets of experiments argue against the possibility that a lysosome localization defect underlies the inability of the PQLC2 P55L/P201L mutant to interact with the C9orf72 complex.
Dear Shawn:

Thank you for submitting your revised manuscript entitled "PQLC2 signals lysosomal cationic amino acid abundance to the C9orf72 complex". We would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below).

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4) Title: The title should be less than 100 characters including spaces. Make the title concise but
While your current title will be appreciated by the specialists, we do not feel that it will be accessible to a broader cell biology audience. Therefore we suggest the following title: "PQLC2 recruits the C9Orf72 complex to lysosomes in response to cationic amino acid starvation"

5) Materials and methods: Should be comprehensive and not simply reference a previous publication for details on how an experiment was performed. Please provide full descriptions (at least in brief) in the text for readers who may not have access to referenced manuscripts. The text should not refer to methods "...as previously described."

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Thank you for this interesting contribution, we look forward to publishing your paper in Journal of Cell Biology.

Sincerely,

Xiaochen Wang
Monitoring Editor
Journal of Cell Biology

Tim Spencer, PhD
Interregnum Executive Editor
Journal of Cell Biology