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Interaction between VPS13A and the XK scramblase is important for VPS13A function in humans

Jae-Sook Park, Yiying Hu, Nancy M. Hollingsworth, Gabriel Miltenberger-Miltenyi and Aaron Neiman
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Original submission

First decision letter

MS ID#: JOCES/2022/260227

MS TITLE: Interaction between VPS13A and the XK scramblase is required to prevent VPS13A disease in humans

AUTHORS: Jae-Sook Park, Yiyeng Hu, Nancy M Hollingsworth, Gabriel Miltenberger-Miltenyi, and Aaron Neiman
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers raise a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

In particular ,I would like you to focus on the quality of the western blots and the quantification provided in support of your claims. In terms of localisation of endogenous proteins, although the criticism of the review is valid, I suggest you:
i. examine the localisation of the protein at the lowest levels of overexpression, which may be more representative of the physiological localisation.
ii. make clear the caveat of this experiment in the main text

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

In this manuscript, Park et al. determined the XK's and VPS13A's regions required to interact with XK and VPS13A. This is important data. However, as the authors acknowledge, all data were obtained by transient-over expression in 293T cells. I do not think that we can determine the localisation of membrane protein by transient-overexpression.

Comments for the author

The authors have a system to analyse the endogenous XK and VPS13A proteins by Western Blotting and access to the patient's red blood cells. Red blood cells express a high level of XK in the plasma membranes. I may recommend determining whether the wild-type and mutant VPS13A protein is present in the membrane fraction of red blood cells with no ER system. Then, use the over-expression system to determine their interaction as the authors acknowledge. My detailed comments are as follows.

1. Title and Abstract: The title and the sentence "---the importance of a different domain in VPS13A in preventing VPS13A disease--" in the abstract may not be appropriate. The different domains are necessary for their function, and mutation in these domains causes disease.
2. In several places, the references are not formatted. {Niemela, 2020 #1226) (Page 5) {Kumar, 2018 #1030} (Page 6).
3. Page 6, line 12; "GPF"-XK should read "GFP"-XK.
4. Figure 1: This Figure shows that the wild-type and mutant VPS13A proteins are expressed in the patient's red blood cells. I understand that human red blood cells express XK at a high level in the plasma membrane. Western blot analysis of the membrane fraction of red blood cells with anti-XK and anti-VPS13A will easily tell whether the wild-type but not mutant VPS13A is present in the plasma membrane.
5. Figure 2B: When membrane proteins are over-expressed, they often undergo aggregation and are stuck at the ER. The localisation of membrane proteins should be examined with endogenous protein or at least in stable transformants.
6. Figure 2C, Page 7, line 5; the authors claim that no VPS13A mutant protein in the immunoprecipitates is not due to the low expression of the mutant because GFP-XK is strongly expressed. I do not understand the logic. I am sure that reducing the total concentration of VPS13 protein will reduce its complex with XK even in a large amount of XK.
7. Figure 2C: There are many bands for GFP-XK. Which one is expected? Please comment on what caused the multiple bands.
8. Figure 3A: Three of the GFP-VPS13A fusion proteins show lower extra bands. Please explain. If they are cleaved GFP, why the size is different among the lanes?
9. Figure 3A: The untagged XK protein behaves as 200 kDa protein in the total lysate when it was co-expressed with VPS13A mutants. It was reduced to 37 kDa in the IP samples. The authors discuss that it may be because the protein in the total lysates was not solubilised. I understand that the sample was heated at 50°C in the presence of SDS. Do the authors think that it is not enough to solubilize XK? Any evidence?
10. Figure 3B: I strongly think that the cellular localisation of membrane proteins (XK in this manuscript) should be studied by stable transformants, not transient over-expression.
11. Figure 4C: The localisation of GFP-XK (patches in the ER) seems to differ with or without VPS13A. Do the authors want to say that XK localises at ER (?) only in the presence of VPS13A? This is again obtained by transient-over expression.
12. Figure 4D: Several bands for GFP-XK. Please explain.
13. Figure 4E: The expression level of GFP-VPS13A3027-3174 is extremely low (less than 10% of the wild-type?). It is difficult to say that the PH region is necessary to interact with XK.

14. Page 11, line 7; The sentence “The XK sequence is largely unstructured except for two short beta strands.” Please define the region of XK. Or, insert “in this region” between “The XK sequence” and “is”.
15. Discussion: The sentence starting “Unlike mutations in the VPS13A VAB domain -----” is not clear.
Please rewrite.
16. Page 13, Line 7; It is unclear why the authors propose “at least two other proteins” is necessary for recruitment to the lipid droplets.

Reviewer 2

Advance summary and potential significance to field

Park et. al. convincingly demonstrate that the PH domain of VPS13A, a protein implicated in chorea-acanthocytosis, is required to form a complex with XK-scramblase, which when overexpressed alters the subcellular localization of VPS13A. The authors also identify the possible region of interaction between the two proteins.

Comments for the author

The results are relevant to the field and present a sufficient advance in knowledge to be published in their present form. The conclusions are generally well supported by experimental data. The difficulties inherent in working with a large, low expressed protein do not allow a level of definition to determine, for example, where these proteins function when expressed at endogenous levels, and whether the contact sites involved are affected by the lack of interaction. Thus, the conclusion stated in the abstract that interaction of VPS13A with XK is necessary for proper localization should be qualified, since " proper localization" is unclear.
The stability of the truncated VPS13A protein does not appear to be greatly affected in the blood of patients. However, when transfected into HEK293T its level is greatly reduced. It cannot be ruled out that the protein levels are also reduced in neurons and, therefore, the conclusion that the C-terminal region is critical for VPS13A disease should also be refined.

Minor comments
Figure 2. Panel B. The absence of colocalization of the mutated protein with ER patches is unclear as the cells depicted do not show ER patches. Also, if these structures form when XK is overexpressed, why are these patches not seen in the rest of the experiments showing a fairly normal ER structure? Does this depend on the use of GFP or Cherry?
XK expression is also evident at the plasma membrane and in a central area that does not colocalize with VPS13A. This should be described and discussed.

Reviewer 3

Advance summary and potential significance to field

Recent work has shown that members of the Vps13/Atg2 family of lipid transporters bind to lipid scramblases but how they interact is not well understood. Previously, the authors showed that VPS13A and the lipid scramblase XK, which are linked to two different diseases that share similar features, form a complex and co-localize when co-expressed in cultured cells. Here, they study this interaction in more detail. They show that some chorea acanthocytosis patients express a mutant form of the VPS13A protein with a C-terminal truncation that disrupts the PH domain. They find that this PH domain is necessary and sufficient for co-localization and co-immunoprecipitation with XK.
Using alphafold prediction and mutational analysis, they map this interaction to a beta-strand of the PH domain and a cytosolic loop in XK.

This work has disease relevance, because it supports the idea that loss of the VPS13A-XK interaction is important for pathogenesis in chorea acanthocytosis and McLeod syndrome. It also presents an advance in terms of basic biology, by contributing to our understanding of the VPS13-

scramblase interaction and demonstrating that the PH domain makes important interactions with a protein partner to influence VPS13 localization. Thus, this work should be of general interest to the field.

Comments for the author

There are some weaknesses and caveats. All experiments rely on overexpressed proteins in HEK293T cells, and the localization of the VPS13A-XK complex at the ER likely results from this overexpression. This caveat is acknowledged by the authors, and because both proteins are normally expressed at very low level, I feel that investigating the endogenous proteins is beyond the scope of this study. It is also worth noting that the results presented in this manuscript are in broad agreement with those presented in a preprint that has not yet been subjected to peer review, and that includes work with endogenous proteins.
My greater concern is with the quality and presentation of some of the data.
Little to no quantitation was provided for any figure. Quantitation of the existing replicates of each experiment would increase confidence in the robustness of the data and should not require new lab work. For many of the microscopy experiments, I found it difficult to know which VPS13A mutants and fragments are localized to ER vs PM vs ring-like ER patches, and the relative frequency of these localization patterns in different experiments, yet these details are important. Quantitation of the localization patterns should be added to many of these experiments, while other co-localization experiments could be quantitated by automated methods (eg. Manders coefficient). Some experiments have additional problems, or could benefit from additional work, as outlined below.
Figure 1:
The patients (esp patient 1) are expected to express only the VPS13A allele with the minor C-terminal truncation, and the results of Fig 2 suggest the truncated allele might be unstable. Thus it is of interest to know if patient lysates have only half (or less) of the level of VPS13A seen in the wild type sample, but this is difficult to discern in the figure perhaps due to uneven loading. Quantitation of VPS13A levels from western blot replicates, normalized to loading controls, should be shown. Note also that “b-actin was used as a loading control” is written twice in the legend.
Figure 2:
In 2B, do the ER circular patches correspond to enlarged mitochondrial contact sites? If not, what organelle is being circled?
In 2C, the very low level of truncated protein in the cell lysates makes this co-IP experiment very unconvincing, and I don’t agree that higher levels of GFP-
XK in the lysate circumvent this problem. If the protein is unstable in the lysate, it could be further degraded during the IP procedure. (Moreover, I expect the higher levels of GFP-XK are due to the transient transfection technique and will vary in replicates). Thus this figure is more suited to a supplement.
Figure 3:
In 3A, XK is shown as an ~200kD protein in the lysate, but only ~40kD in the IP sample. However, the 37kD region of the lysate gel seems to be cut off, and should also be shown. Note also that the aberrant migration in SDSPAGE could be caused by heating samples (eg at 50C as was done here), poor solubilization or use of sonication. Ryoden et al solubilize XK lysates in sample buffer at room temperature for 2h and do not sonicate, and show a ~40kD band in cell lysate thus it seems likely that the aberrant migration could be fixed.
Figure 5:
LDDT scores and PAE plots should be provided to support the Alpha fold predictions. Details of the alphafold analysis should be provided in the Methods.
Figure 6:
Is the location of the cytosolic leaflet of the membrane properly placed with respect to the XK structure? It is my understanding that most of the XK alpha helices are embedding in the membrane.

First revision

Author response to reviewers' comments

Reviewer 1 Comments for the Author:
My detailed comments are as follows.

1. Title and Abstract: The title and the sentence "---the importance of a different domain in VPS13A in preventing VPS13A disease--" in the abstract may not be appropriate. The different domains are necessary for their function, and mutation in these domains causes disease.

-The title and abstract have been edited as suggested

2. In several places, the references are not formatted. {Niemela, 2020 #1226) (Page 5) {Kumar, 2018 #1030} (Page 6).

-Corrected

3. Page 6, line 12; "GPF"-XK should read "GFP"-XK.

-Corrected

4. Figure 1: This Figure shows that the wild-type and mutant VPS13A proteins are expressed in the patient's red blood cells. I understand that human red blood cells express XK at a high level in the plasma membrane. Western blot analysis of the membrane fraction of red blood cells with anti-XK and anti-VPS13A will easily tell whether the wild-type but not mutant VPS13A is present in the plasma membrane.

-We thank the reviewer for the suggestion. Unfortunately, we do not have sufficient material in the patient samples available to allow fractionation of the cells.

5. Figure 2B: When membrane proteins are over-expressed, they often undergo aggregation and are stuck at the ER. The localisation of membrane proteins should be examined with endogenous protein or at least in stable transformants.

-We agree with the reviewer about the difficulties of working with overexpressed membrane proteins. We reduced XK expression by making a deletion in the CMV promoter. This deletion lowered GFP-XK expression more than 10 fold and reduced the fluorescence intensity in transfected cells but did not change the patterns of localization seen with VPS13 co-transfection (new Supplemental Figure 1). This does not resolve the question of where XK is found at native expression level and we have tried to emphasize this caveat in the text. We explicitly say in the text that we are not using the localization patterns to infer where the proteins normally localize but simply as an assay for their association in vivo.

6. Figure 2C, Page 7, line 5; the authors claim that no VPS13A mutant protein in the immunoprecipitates is not due to the low expression of the mutant because GFP-XK is strongly expressed. I do not understand the logic. I am sure that reducing the total concentration of VPS13 protein will reduce its complex with XK even in a large amount of XK.

-We thank the reviewer for highlighting this issue. In response, we have repeated the experiment using the additional control of the VPS13-W2460R allele. In earlier work, we showed that W2460R lowered the steady-state level of the protein in HEK293T cells but did not interfere with the co-immunoprecipitation. In the modified Figure 2C it is now shown that, while both the W2460R and 31ΔC mutations lower VPS13A levels to similar extents, the co-precipitation of the 31ΔC protein is >10-fold reduced relative to both WT and W2460R. These new data are consistent with the idea that the 31ΔC mutation is distinct from W2460R in disrupting the interaction between VPS13A and XK.

7. Figure 2C: There are many bands for GFP-XK. Which one is expected? Please comment on what caused the multiple bands.

-The slower mobility forms are likely caused by aggregation/incomplete solubilization of the multiple hydrophobic transmembrane regions of XK. This is now noted in the legend to Figure 2.

8. Figure 3A: Three of the GFP-VPS13A fusion proteins show lower extra bands. Please explain. If they are cleaved GFP, why the size is different among the lanes?

-These are likely degradation products, though based on size, not likely simple cleavages of the VPS13A sequence from GFP.

9. Figure 3A: The untagged XK protein behaves as 200 kDa protein in the total lysate when it was co- expressed with VPS13A mutants. It was reduced to 37 kDa in the IP samples. The authors discuss that it may be because the protein in the total lysates was not solubilised. I understand that the sample was heated at 50°C in the presence of SDS. Do the authors think that it is not enough to solubilize XK? Any evidence?

-As part of repeating the immunoprecipitations we have carefully standardized the solubilization procedure and find that the mobility of XK is more reproducible between experiments and samples. It appears that at higher temperatures (above 50°C) solubization is worse, as has been noted before for multipass transmembrane proteins. The patterns of mobility for XK and GFP-XK are now consistent between IPs and total lysates as well as between different figures.

10. Figure 3B: I strongly think that the cellular localisation of membrane proteins (XK in this manuscript) should be studied by stable transformants, not transient over-expression.

-See reply to comment 5 above.

11. Figure 4C: The localization of GFP-XK (patches in the ER) seems to differ with or without VPS13A. Do the authors want to say that XK localizes at ER (?) only in the presence of VPS13A? This is again obtained by transient-over expression.

-Indeed, in our earlier study we showed that the appearance of concentrated patches of XK in the ER is induced by co-expression with VPS13A and these are likely artifacts of the overexpression of both proteins. Moreover, formation of these patches requires the N-terminus of VPS13A (Figure 3). Nonetheless, the appearance of these structures is evidence of association in vivo and is lost when the VPS13A-31ΔC and XK-6A mutants are used.

12. Figure 4D: Several bands for GFP-XK. Please explain.

-See reply to comment 8, above

13. Figure 4E: The expression level of GFP-VPS13A3027-3174 is extremely low (less than 10% of the wild-type?). It is difficult to say that the PH region is necessary to interact with XK.

-The expression level of constructs varies with the transfection efficiency on different days. We have repeated this experiment and replaced the figure with one in which the expression of GFP-VPS13A3027-3174 is comparable in transfections with both XK and XK-6A. The result remains the same (greatly reduced co-precipitation of XK-6A).

14. Page 11, line 7; The sentence “The XK sequence is largely unstructured except for two short beta strands.” Please define the region of XK. Or, insert “in this region” between “The XK sequence” and “is”.

-Corrected

15. Discussion: The sentence starting “Unlike mutations in the VPS13A VAB domain ---
--” is not clear. Please rewrite.

-This passage has been rewritten.

16. Page 13, Line 7; It is unclear why the authors propose “at least two other proteins” is necessary for recruitment to the lipid droplets.

-This section has been clarified.

Reviewer 2 Advance Summary and Potential Significance to Field:
Park et. al. convincingly demonstrate that the PH domain of VPS13A, a protein implicated in chorea-acanthocytosis, is required to form a complex with XK-scramblase, which when overexpressed alters the subcellular localization of VPS13A. The authors also identify the possible region of interaction between the two proteins.

Reviewer 2 Comments for the Author:
1. The results are relevant to the field and present a sufficient advance in knowledge to be published in their present form. The conclusions are generally well supported by experimental data. The difficulties inherent in working with a large, low expressed protein do not allow a level of definition to determine, for example, where these proteins function when expressed at endogenous levels, and whether the contact sites involved are affected by the lack of interaction. Thus, the conclusion stated in the abstract that interaction of VPS13A with XK is necessary for proper localization should be qualified, since " proper localization" is unclear.

-The reviewer’s point is well taken and we have removed this conclusion from the abstract

2. The stability of the truncated VPS13A protein does not appear to be greatly affected in the blood of patients. However, when transfected into HEK293T its level is greatly reduced. It cannot be ruled out that the protein levels are also reduced in neurons and, therefore, the conclusion that the C-terminal region is critical for VPS13A disease should also be refined.

-This conclusion has been softened in the text.

Minor comments
-3. Figure 2. Panel B. The absence of colocalization of the mutated protein with ER patches is unclear as the cells depicted do not show ER patches. Also, if these structures form when XK is overexpressed, why are these patches not seen in the rest of the experiments showing a fairly normal ER structure? Does this depend on the use of GFP or Cherry?

-The co-expression with VPS13A induces the bright patches of ER localized XK and vice versa. This has been clarified in the text (p.6)

4. XK expression is also evident at the plasma membrane and in a central area that does not colocalize with VPS13A. This should be described and discussed.

-A description has been added (p.6)

Reviewer 3 Comments for the Author:
There are some weaknesses and caveats. All experiments rely on overexpressed proteins in HEK293T cells, and the localization of the VPS13A-XK complex at the ER likely results from this overexpression. This caveat is acknowledged by the authors, and because both proteins are normally expressed at very low level, I feel that investigating the endogenous proteins is beyond the scope of this study. It is also worth noting that the results presented in this manuscript are in broad agreement with those presented in a preprint that has not yet been subjected to peer review, and that includes work with endogenous proteins.

1. My greater concern is with the quality and presentation of some of the data. Little to no quantitation was provided for any figure. Quantitation of the existing replicates of each experiment would increase confidence in the robustness of the data and should not require new lab work. For many of the microscopy experiments, I found it difficult to know which VPS13A mutants and fragments are localized to ER vs PM vs ring-like ER patches, and the relative frequency of these localization patterns in different experiments, yet these details are important. Quantitation of the localization patterns should be added to many of these experiments, while other co-localization experiments could be quantitated by automated methods (eg. Manders coefficient). Some

experiments have additional problems, or could benefit from additional work, as outlined below.
-Quantification for several of these localizations was provided in the text in the original version. This information has been moved to the Figure Legends to make it easier to find. In addition to this, we have added quantification for the localization patterns seen in Figures 3B and 5B. Also we have added quantification for the relative amounts of wild type and mutant VPS13A proteins that co-precipitate with XK (Figure 2C).

2. Figure 1:
The patients (esp patient 1) are expected to express only the VPS13A allele with the minor C-terminal truncation, and the results of Fig 2 suggest the truncated allele might be unstable. Thus it is of interest to know if patient lysates have only half (or less) of the level of VPS13A seen in the wild type sample, but this is difficult to discern in the figure perhaps due to uneven loading. Quantitation of VPS13A levels from western blot replicates, normalized to loading controls, should be shown. Note also that "b-actin was used as a loading control" is written twice in the legend.

-We thank the reviewer for the suggestion. Unfortunately, the film exposures we have for these blots could not be reliably quantified and we do not have sufficient patient material to perform additional Westerns. We cannot say, therefore, what the mutant protein level is relative to wild type, and we have edited the text to reflect this limitation of our data. The critical point that is supported by our data is that the C-terminally truncated protein is detectable, unlike other reported disease alleles.

3. Figure 2:
In 2B, do the ER circular patches correspond to enlarged mitochondrial contact sites? If not, what organelle is being circled?

-In our earlier study, we found that the ER circular patches do not correspond to ER surrounding another organelle. In the bioRxiv paper from the DeCamilli lab cited by the reviewer, they use correlative light/electron microscopy to show that these are whorls of ER membrane.

4. In 2C, the very low level of truncated protein in the cell lysates makes this co-IP experiment very unconvincing, and I don't agree that higher levels of GFP-XK in the lysate circumvent this problem. If the protein is unstable in the lysate, it could be further degraded during the IP procedure. (Moreover, I expect the higher levels of GFP-XK are due to the transient transfection technique and will vary in replicates). Thus this figure is more suited to a supplement.

-We thank the reviewer for highlighting this issue, as did reviewer #1. We have added additional data using another VPS13A allele, which also shows lowered expression and quantified the effects on the co-IP (see reply to reviewer #1 comment #6). We feel this significantly strengthens the original conclusion.

5. Figure 3:
In 3A, XK is shown as an ~200 kD protein in the lysate, but only ~40kD in the IP sample. However, the 37kD region of the lysate gel seems to be cut off, and should also be shown. Note also that the aberrant migration in SDSPAGE could be caused by heating samples (eg at 50C as was done here), poor solubilization or use of sonication. Ryoden et al solubilize XK lysates in sample buffer at room temperature for 2h and do not sonicate, and show a ~40kD band in cell lysate thus it seems likely that the aberrant migration could be fixed.

-All of the Western blots have been repeated to better standardize the solubilization of XK. See response to Reviewer #1, comment #9

6. Figure 5:
LDDT scores and PAE plots should be provided to support the Alpha fold predictions. Details of the alphafold analysis should be provided in the Methods.

-The requested information has been added as Supplementary Figure 2. A more complete description of the Alphafold procedure has been added to the Methods.

7. Figure 6:
Is the location of the cytosolic leaflet of the membrane properly placed with respect to the XK structure? It is my understanding that most of the XK alpha helices are embedding in the membrane.

-The reviewer is correct about the XK alpha helices. The position of the line indicating the inner leaflet has been adjusted in a modified figure to more closely approximate the position of the leaflet.

Second decision letter

MS ID#: JOCES/2022/260227

MS TITLE: Interaction between VPS13A and the XK scramblase is important for VPS13A function in humans

AUTHORS: Jae-Sook Park, Yiying Hu, Nancy M Hollingsworth, Gabriel Miltenberger-Miltenyi, and Aaron Neiman
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers gave favourable reports but some critical points from Reviewer 1 have been overlooked. These need to be addressed in the final version of your manuscript either by providing the requested data or by demonstrating that these avenues of investigation have been unsuccessful. I hope that you will be able to carry these out because this would be the last round of revision allowed.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

In this manuscript, the authors determined the regions where XK and VPS13A interact. This information will be useful for the field.

Comments for the author

The localization of XK-VPS13A was studied by transient-over-expression. I commented that the localization of membrane proteins should be examined with endogenous proteins or at least with stable transformants. This comment was neglected. I also suggested checking the localization of the XK-VPS13A with red blood cells.

The authors responded that they could not obtain cells sufficient for fractionation. I am puzzled! Red blood cells do not have intracellular organelles. We do not have to fractionate them to get the plasma membranes.
Antibodies recognizing the endogenous XK and VPS13A proteins are commercially available, too.

Reviewer 2

Advance summary and potential significance to field

The authors answered all my questions satisfactorily. I have no further comments.

Comments for the author

The authors answered all my questions satisfactorily. I have no further comments.

Reviewer 3

Advance summary and potential significance to field

Recent work has shown that members of the Vps13/Atg2 family of lipid transporters bind to lipid scramblases but how they interact is not well understood. Previously, the authors showed that VPS13A and the lipid scramblase XK, which are linked to two different diseases that share similar features, form a complex and co-localize when co-expressed in cultured cells. Here, they study this interaction in more detail. They show that some chorea acanthocytosis patients express a mutant form of the VPS13A protein with a C-terminal truncation that disrupts the PH domain. They find that this PH domain is necessary and sufficient for co-localization and co-immunoprecipitation with XK.
Using alphafold prediction and mutational analysis, they map this interaction to a beta-strand of the PH domain and a cytosolic loop in XK.

This work has disease relevance, because it supports the idea that loss of the VPS13A-XK interaction is important for pathogenesis in chorea acanthocytosis and McLeod syndrome. It also presents an advance in terms of basic biology, by contributing to our understanding of the VPS13-scramblase interaction and demonstrating that the PH domain makes important interactions with a protein partner to influence VPS13 localization. Thus, this work should be of general interest to the field.

Comments for the author

The revised manuscript adequately addresses my concerns, and I consider it to be suitable for publication.

Second revision

Author response to reviewers' comments

Reviewer #1 has two remaining concerns that he/she felt were not properly addressed in the revision. I apologize that the rebuttal letter was not clear on these points. The specific comments are that:

a) the localization of membrane proteins should be examined with endogenous proteins or at least with stable transformants;
b) checking the localization of the XK-VPS13A with red blood cells.

To the first point, as we noted, the endogenous levels of both proteins are below cytological detection (this is an issue the whole field is struggling with at the moment). The use of stable transfectants does not help this situation as the integrated constructs would still need to be overexpressed. As we demonstrated in the new supplementary data, even a > 10 fold reduction in expression of the transient transfection did not alter our results. More importantly, we are using localization simply as an assay for interaction and not making any claims as to the localization of the proteins at their native levels. This point is addressed explicitly in the text. Therefore the significant work required to construct and examine stable transfectants would not alter the conclusions of the paper.

On the second point, I misunderstood the reviewer's concern. The original review suggested determining if the "wild-type and mutant VPS13A protein is present in the membrane fraction of red blood cells", thus our response mentioned "fractionation". I believe the reviewer meant a membrane vs. cytoplasmic fraction of the red blood cells not subfractionation of the membranes. As I noted, we do not have material available for this experiment with patient samples. However, it has been previously reported that both VPS13A and XK are detectable by Western blot in preparations of erythrocyte membranes and that the amount of VPS13A in this fraction is reduced in samples from patients lacking XK (Urata et al; 2019). In the re-revised manuscript I have added this fact and the reference to the Discussion. The precise role of VPS13A and XK in red blood cells is an important question but, again, beyond the scope of the present study.

Third decision letter

MS ID#: JOCES/2022/260227

MS TITLE: Interaction between VPS13A and the XK scramblase is important for VPS13A function in humans

AUTHORS: Jae-Sook Park, Yiying Hu, Nancy M Hollingsworth, Gabriel Miltenberger-Miltenyi, and Aaron Neiman
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in the Journal of Cell Science, pending standard ethics checks. I am particularly grateful for your reply to the final concernsof Reviewer 1. This is key for the JCS transparent approach to refereeing.