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## **Genetically encoded impairment of neuronal KCC2 cotransporter function in human idiopathic generalized epilepsy**

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### **Review timeline:**

Submission date:	27 March 2014
Editorial Decision:	14 April 2014
Revision received:	18 April 2014
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### **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.)

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### **Transfer Note:**

This manuscript was transferred from another journal with anonymous referee reports. Given the reports and the related paper from Kaila's group that we have just published, we decided to contact a single referee only.

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Editor: Esther Schnapp

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1st Editorial Decision

14 April 2014

Thank you for the submission of your manuscript to EMBO reports. We have now received the comments from the referee who was asked to assess it, and I am happy to tell you that s/he supports publication of the study in EMBO reports.

Can you please address all the comments by this referee and send us a revised manuscript as soon as possible? Please also shorten the manuscript text, as the character count largely exceeds our limit. The revised manuscript may not exceed 30,000 characters (including spaces, references and figure legends) and 5 main plus 5 supplementary figures, which should directly relate to a corresponding main figure. The Results and Discussion sections can be combined, which may help to eliminate some redundancy that is inevitable when discussing the same experiments twice. Commonly used materials and methods can further be moved to the supplementary information, but please note that materials and methods essential for the understanding of the experiments described in the main text must remain in the main manuscript file.

Regarding data quantification, can you please specify the bars and error bars for figure 5B?

I look forward to receiving a revised version of your manuscript as soon as possible.

#### REFEREE REPORT:

##### Referee #1:

In this manuscript Kahle et al. provide evidence that KCC2 variants may contribute to idiopathic generalized epilepsy (IGE), while Pusjarkov et al. reported that R952H might be associated with febrile seizures. Kahle et al. reports on a second C-terminal variant (R1049C) in addition to R952H in patients with IGE. Only after increasing the number of controls, the association for one of the variants (R1049C) becomes significant ( $p = 0.044$ ), while R952H comes close to significance ( $p = 0.065$ ). Taken together the  $p$  value for C-terminal KCC2 variants (i.e. R952H and R1049C) becomes significant.

Although the authors have been partially scooped by Pusjarkov et al., the current manuscript strengthens the genetic link between epilepsy and KCC2.

##### Minor points:

Overall one can feel that the manuscript was written in a hurry!

Page 5 "Nevertheless, R952H is enriched in IGE cases when population stratification is addressed". What does this exactly mean?

Abstract: Is severe IGE correct? This is not reported in the description of the cohort in the "Materials and Methods" section.

Figure 2C,D: The labeling of significance levels is puzzling. What has been compared?

Figure 3: From the "Materials and Methods" section the description how surface expression and overall expression can be distinguished is difficult to follow for the uninformed reader.

Page 10: I would prefer to display the Western blots to demonstrate that total KCC2 expression is indeed similar to WT for both variants. For the analysis of the phosphorylation all individual blots which were used for the quantification should be displayed in the supplement.

References: Some of the references need to be edited!

1st Revision - authors' response

18 April 2014

##### Response to editor's comment:

Can you please address all the comments by this referee and send us a revised manuscript as soon as possible? Please also shorten the manuscript text, as the character count largely exceeds our limit. The revised manuscript may not exceed 30,000 characters (including spaces, references and figure legends) and 5 main plus 5 supplementary figures, which should directly relate to a corresponding main figure. The Results and Discussion sections can be combined, which may help to eliminate some redundancy that is inevitable when discussing the same experiments twice. Commonly

used materials and methods can further be moved to the supplementary information, but please note that materials and methods essential for the understanding of the experiments described in the main text must remain in the main manuscript file.

*We have drastically reduced the amount of text from > 49,000 to almost 30,000 characters (including spaces, references and figure legends). To do this, we have eliminated superfluous information, shortened the introduction and discussion, and moved a considerable amount of methods/materials (including standard techniques like gramicidin perforated patch experiments, Western blotting, etc.) to a Supplementary Methods and Materials section. The text and figure legends describe methods details in sufficiently for a good understanding of the paper, and the reader is directed to the Supplementary section when appropriate for further methodological detail.*

Regarding data quantification, can you please specify the bars and error bars for figure 5B?

*This has been clarified in Figure 5.*

Response to referee

Referee #1:

In this manuscript Kahle et al. provide evidence that KCC2 variants may contribute to idiopathic generalized epilepsy (IGE), while Pusjarkov et al. reported that R952H might be associated with febrile seizures. Kahle et al. reports on a second C-terminal variant (R1049C) in addition to R952H in patients with IGE. Only after increasing the number of controls, the association for one of the variants (R1049C) becomes significant ( $p = 0.044$ ), while R952H comes close to significance ( $p = 0.065$ ). Taken together the  $p$  value for C-terminal KCC2 variants (i.e. R952H and R1049C) becomes significant.

Although the authors have been partially scooped by Pusjarkov et al., the current manuscript strengthens the genetic link between epilepsy and KCC2.

Minor points:

Overall one can feel that the manuscript was written in a hurry!

Page 5 "Nevertheless, R952H is enriched in IGE cases when population stratification is addressed". What does this exactly mean?

*This sentence has been changed to "Nevertheless, R952H is enriched in these Quebec IGE cases compared to Quebec controls." We were trying to address the fact that despite R925H has a higher frequency in the Quebec population compared to other reported frequencies (in EVS) it is still enriched in the Quebec IGE cohort compared to the corresponding population controls.*

Abstract: Is severe IGE correct? This is not reported in the description of the cohort in the "Materials and Methods" section.

*"Severe" is not precise and has been removed; the definition of our IGE cases is provided in Supplementary Data*

Figure 2C,D: The labeling of significance levels is puzzling. What has been compared?

*The significance bar with 4 prongs compares WT, R1049C, and R952H relative to mock-transfected controls; the significance bar with 3 prongs compares R1049C and R952H to WT.*

Figure 3: From the "Materials and Methods" section the description how surface

expression and overall expression can be distinguished is difficult to follow for the uninformed reader.

*This has been clarified in the appropriate section that has also been moved to Supplementary Materials and Methods to meet the character limit of 30,000.*

Page 10: I would prefer to display the Western blots to demonstrate that total KCC2 expression is indeed similar to WT for both variants. For the analysis of the phosphorylation all individual blots which were used for the quantification should be displayed in the supplement.

*-See Figure 5A showing total KCC2 expression is the same for both KCC2 IGE variants.  
-We feel the representative blots displayed in the figures, coupled with the bar graph summaries (e.g., 5a and 5b) is sufficient, the standard means of presentation, and how we have displayed this data in the past using the same antibody and techniques (Lee et al., 2007, JBC; Lee et al., 2011, Nat Neurosci).*

References: Some of the references need to be edited!

*Agreed. We have updated all references given the drastic cutting in words to meet the word limit (47,000 to 30,000 characters)*

2nd Editorial Decision

23 April 2014

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I am pleased to accept your manuscript for publication in the next available issue of EMBO reports.

Thank you for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.