Reviewer's report

Title: Absence of Chloride Intracellular Channel 4 (CLIC4) Predisposes to Acute Kidney Injury But Has Minimal Impact on Recovery

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Reviewer: David Long

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Edwards and colleagues examine the role of chloride intracellular channel 4 in healthy and diseased mouse kidneys. They demonstrate that mice lacking Clic4 have fewer glomeruli, a less dense peritubular capillary network and increased proteinuria. Furthermore, knock-out of Clic4 led to increased susceptibility to folic-acid induced acute kidney injury but the recovery from this insult was not altered. Overall, the study is clearly described, has been performed well and provides novel information into the role of Clic4 in the normal and diseased kidney. However, I have a few issues for the authors to resolve as outlined below.

Major

1) The authors demonstrate that glomerular number and endothelial density are reduced in Clic4 knock-out mice and speculate that this may be due to decreased angiogenesis. It would strengthen the article if the authors were able to demonstrate that endothelial proliferation/apoptosis was altered to show changes in active angiogenesis. Are there are changes in the expression of genes which regulate vascular growth/remodelling such as VEGF-A or angiopoietins?

2) Clic4 knock-out mice have alterations in proteinuria (Figure 5) as determined by protein/creatinine measurements in the urine. Ideally, as up to one half of urinary creatine in mice is derived from tubular secretion (Eisner et al Kidney Int 2010 77: 519-526), 24-hour urine collection should be made in metabolic cages; this should be mentioned as a limitation of the study. The authors should also describe recent data showing that lack of glomerular number can directly lead to proteinuria in mice (Long et al Kidney Int 201 83, 1118–1129.

3) Clic4 knock-out mice are then challenged with folic acid to induce acute tubular necrosis. The Clic4 null mice were more susceptible to folic-acid injury with higher blood urea nitrogen at 48 hours after injection. Were there also any changes in the protein excretion as seen in the earlier non-diseased mice? Also, were there any differences observed between genders?

4) As mentioned in the Discussion a potential explanation for the finding that folate injury is more pronounced in Clic4 mice is that endothelial repair mechanisms are less effective due to their being a deficiency in peritubular capillary density. As this was one of the main findings in the unchallenged Clic4
null mice, the potential role of angiogenesis in acute kidney injury and the folate model should be described in more detail in the Introduction and Discussion (see for example Long et al Kidney Int 2008 74: 300-309). It would be of significant interest to examine changes in endothelial turnover and vascular genes following folic acid injury in wild-type and Clic4 knock-outs.

Minor

1) Page 11, statistics, a short explanation should be provided as to why the different statistical tests were used
2) page 11, results, -please clarify age of animals examined for Clic4 immunostaining.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

No