Reviewer’s report

Title: Complexity of the 5’UTR region of the CLCN5 gene: eleven 5’UTR ends are differentially expressed in the human kidney

Version: 1 Date: 9 April 2014

Reviewer: Jonathan Lippiat

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This manuscript describes 5’ transcript variants of the CLCN5 gene. CLCN5 is responsible for the majority of Dent’s disease cases (Dent’s I). Previous studies have focussed on inherited variations in the coding regions of the gene, which disrupt protein structure. Many Dent’s disease cases have yet to have a genetic description, which means that either different genes are involved (e.g. OCRL1 – Dent’s II) or non-coding regions of the CLCN5 gene are affected.

Major Compulsory Revisions
None

Minor Essential Revisions
1. Abstract requires some editing: delete “by real time PCR” or rewrite as “most abundant isoforms, detected by real time PCR, contain exon ....”; rewrite “classic 747 amino acid” as “canonical 747 amino acid” (also p.17, but confirm if the canonical sequence contains 747 (Abstract) or 746 (Discussion) amino acids).
2. Methods require editing: delete “After” to read “Successful amplification was confirmed…” on p.6.
3. More detail required in the ENCODE bioinformatics analysis: from which genome (cell line?) were ChIP data analysed?
4. The results from the bioinformatics analysis are presented in the Discussion. These need to be described as a distinct section in the Results and interpreted in the Discussion.
5. The mutation in the putative 5’ UTR previously described in Tosetto et al. (2009) should be again evaluated in the Discussion. This mutation involves exon Ib, which, according to this manuscript (p. 17), is included in several transcripts, and in particular those that extend the N-terminal primary structure. Can any further ideas relating to the effects of this mutation be generated by reference to these particular transcripts?
6. Figure 5 and legend: Change $1.00 \times 10^0$ numerical format to $10^0$ or log values.

Discretionary Revisions
7. I believe that it would not be incorrect to present images exported from the UCSC Genome Browser showing exons and the overlayed tracks described in
the manuscript to illustrate the location of the transcriptionally-active regions of the CLCN5 gene. This should be considered by the authors as an additional figure in the main manuscript or supplementary data.

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

**Quality of written English:** Needs some language corrections before being published

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

**Declaration of competing interests:**

I declare that I have no competing interests.