Reviewer’s report

Title: Expression of a novel short isoform of the kidney disease protein podocin in human kidney

Version: 1 Date: 17 October 2012

Reviewer: Maddalena Gigante

Reviewer’s report:

Dr. Völker and colleagues produced an interesting manuscript which provides new evidences on the protein level of the expression in human kidneys of a shorter isoform of podocin, lacking of exon 5 encoding the central part of the PHB-domain, and supports the previously published data by Horinouchi et al. (Kidney International, 2003; 64: 2092–2099) and Relle et al. (Modern Pathology, 2011; 24: 1101–1110). The question posed by the authors is quite well defined; the methods are appropriate; the data seems sound and the writing acceptable. Anyway, some aspects of discussion and conclusion sections are not adequately supported by the result data and the title does not accurately convey the manuscripts’ founding. The manuscript would be considerably stronger after addressing some points.

- MAJOR COMPULSORY REVISIONS

1. A figure showing the immunoprecipitation results (podocin-binding proteins) should be added.

2. The larger canonical isoform of podocin should be also subjected to mass spectrometry and the basepeak chromatogram should be reported. The differences of tryptic peptide analysis between canonical and short isoforms should be showed and marked.

- MINOR ESSENTIAL REVISIONS

• TITLE

1. The word “novel” should be avoid: the data showed by Dr. Relle and colleagues (Modern Pathology, 2011; 24: 1101–1110) using RT–polymerase chain reaction and immunoblotting followed by sequence analysis, are the real first evidence to prove the expression of a “novel” podocin isoform.

2. Podocin is a “kidney protein” not a “kidney disease protein”.

• METHODS

3. Reagents and Plasmids section: The authors should report at least the name of kit used for site direct mutagenesis.

4. Immunoprecipitation section: The origin of human glomeruli samples should be reported;
5. Immunoprecipitation section: The expected molecular weight of the short isoform should be specified;

6. Immunoprecipitation section: “CID” definition should be reported in the List of Abbreviations

• RESULTS AND DISCUSSION, THIRD PARAGRAPH

7. The authors state:
“The low amount of starting material for MS analyses resulted in elution of the mass 1078.2191 for about 15 seconds (Fig. 2C)”. Anyway, the elution peak of m/z 1078.2191 reported in figure 2 was at 40.02 minutes. Could you please clarify this point?

8. The authors state:
“Taking the high mass accuracy of m/z 1078.2191 with the predicted peptide into account and the fact that the corresponding MS/MS spectrum could only be found in Podshort, we report initial evidence on the protein level for the existence of a shorter isoform of human podocin (Fig. 2B)”.

Figure 2B shows the basepeak chromatogram of the eluting peptides from the specific band of short podocin isoform, thus I think that the previous authors’ statement referring to figure 2 should be avoid. This sentence should be restated if mass spectrometry analysis of canonical isoform is not reported.

• FIGURES

9. Figure 3C: the authors should indicate in figure legend what are lane 1, 2, 3, …etc.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

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