Reviewer’s report

Title: Identification of the soluble form of tyrosine kinase receptor Axl as a potential biomarker for intracranial aneurysms rupture

Version: 1 Date: 5 November 2014

Reviewer: Joe Moxon

Reviewer’s report:

Dear Editor

Many thanks to you and the authors for the opportunity to review this paper. Dr Xu and colleagues describe a study aimed at identifying biomarkers for ruptured aortic aneurysm utilising proteomic analyses to profile the CSF taken from several patient groups, followed by validation in a larger independent cohort. The authors suggest that soluble Axl may have potential to aid patient stratification. In all, the paper is of interest however I have several reservations which prevent publication in its current state. Specifically:

Major Compulsory Revisions

1) The authors do not show any proteomic data to support their results or conclusions. Ideally, a supplement detailing the peptides used to identify the proteins should be submitted as an online supplement, or a link to a data repository should be supplied. The proteomics press provide details of expected reporting standards, and the authors are referred to the statement by Molecular and Cellular Proteomics: http://www.mcponline.org/site/misc/ParisReport_Final.xhtml. Please revise and adhere to these standards.

2) Was only 1 iTRAQ experiment (i.e. 1 batch of labelled peptides) run? If so, please state. If not, please describe inter-experiment variation etc.

3) I am a little unclear on the statistical methods used to compare protein expression between treatment groups. The authors mention the application of ANOVA or Kruskal-Wallis tests, however, it is not immediately clear when these are employed. I would like to see a more explicit description of how the stats are used. Moreover, the authors mention the use of multiple testing corrections, but again, it is not clear to what data these are applied. The proteomic data would require extensive FDR correction, however I cannot see whether this has been used here. Finally, I would like to see FDR corrected p-values included in the tables for all of the proteins suggested to be differentially expressed to provide further confidence in the data.

4) I appreciate the difficulties associated with writing in a second language, however, the wording of the current draft is not easy to follow. Consequently, I am a little confused by some of the points made by the authors. It therefore
becomes difficult to assess the accuracy of the data interpretation. For example, page 11 states “However, if a protein, e.g., nectin-like protein 2 (IPI IPI00003813) displayed a significant decrease in RIA (iTRAQ ratio is 0.61 ± 0.13) but an increase in UIA (iTRAQ ration is 1.62 ± 0.23), we would not only consider this protein unique to RIA but also to UIA”. I am not sure how something can be considered unique to 2 things?

In all the manuscript contains a number of convoluted and ambiguous sentences - please reword extensively for clarity.

Minor essential revisions

a) Methods section: Please provide a little more detail on how patients were selected to the discovery or validation stages? E.g. were they consecutively recruited, or was further sorting done?

b) Pg 8 – please correct ‘Six SCX fractionated peptides’ to ‘Six SCX peptide fractions’

c) The following sections would be better included in the methods, not the results:
   • Pg 10 sentence starting ‘To further illustrate…’,
   • Pg 12 sentence starting ‘As the first step….’

d) Pg 12 – section starting ‘Accumulating evidence indicates…’ should go in the discussion.

e) Pg 12 – Please provide concentrations (e.g. median and IQR) for plasma Axl, in addition to ratios.

f) supplementary file 1 – please provide better clarification of the headings in the table. For example, what do ‘Hunt’ and ‘Fishe’ mean?

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

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests