Reviewer's report

Title: Protein modeling to assess the pathogenicity of rare variants of SERPINA1 in patients suspected of having Alpha 1 Antitrypsin Deficiency

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Reviewer: Annamaria Fra

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In this work, NGS sequencing of the SERPINA1 gene was applied to a US cohort of patients with suspected AATD, based on low AAT plasma levels and on clinical presentation. This analysis led to identify rare SERPINA1 alleles in several cases. The authors then used a set of computational methods to predict the pathogenic potential of the SNPs identified. Indeed, the discovery of many novel SERPINA1 variations calls for the implementation of novel diagnostic strategies to timely assess their pathological significance. However, the use of predictive algorithms, generally developed on large datasets, may be inconsistent when applied to a specific gene without any experimental validation, and should be critically evaluated.

I suggest a revision of text and figures before the paper is considered for publication.

Here are some suggestions to improve data presentation:

-most of the AAT variations found in this study have been already reported and some are well characterised (eg. In PMID 24084503; 29882371; 29232161; 22330941; 20453271; 22008137; 2696185; 26270547). Computational predictors have been also used and evaluated previously on SERPINA1 variants (eg in 29882371; 27296815). Although the presentation of new cases is certainly helpful to establish an association between such very rare AAT alleles and the disease, reference to previous work should be clear and the new data discussed in view of previous findings.

-The AAT levels are better presented along with CRP values, as an inflammatory state could explain normal AAT values in the presence of deficient alleles (eg "There was only one exception from the general agreement between computational predictions and AAT serum levels - the P369H mutation, observed in patient 21034. All three computational analyses predicted the mutation to be highly deleterious; however, the AAT serum level was normal (121.2 mg/dL)").

-The IEF results are mentioned in the text but not shown.

-The novel variations should be clearly identified, eg by listing them in a separate table, and discussed in details. The outcome of null alleles is pretty clear in the field, but novel amino acid substitutions would benefit from a specific structural evaluation. To my knowledge, novel variants are: I9N (in the leader sequence and therefore unlikely to have an effect; here numbering is not consistent with the conventional nomenclature adopted in this paper, as evident in Figure E1 also for I50N); P28L; Q40R; M221T and A142D. A more focused discussion of these AAT variations, their structural features and their prediction scores, would allow to appreciate the performance and possible flaws of the different predictors used, when confronted with truly unknown cases.
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Unable to assess

Are the conclusions drawn adequately supported by the data shown?
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No

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